cell density in the nbM and an absolute reduction in cell number of 75 percent ($P < .001$). No consistent alterations in cell density were observed in adjacent structures contained within the sections such as the dorsal globus pallidus or the hypothalamus. The dbB, present in some sections, also showed loss of nerve cells. Thus, these pathological findings indicate that the cholinergic deficits in the cortex and the hippocampus of patients dying with AD result from a degeneration of nerve cells in the nbM.

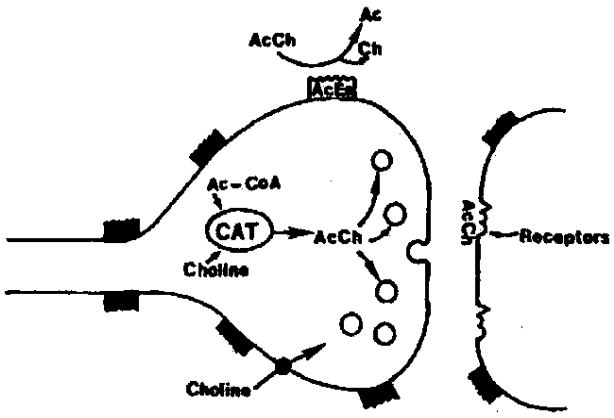
More recently, we have shown that cholinergic innervation is important in the evolution of neuritic plaques. Since aged monkeys, like humans, may develop neuritic plaques with increasing age (*47*), we used silver techniques to reveal the argentophilic neurites, Congo red to stain amyloid, and AChE histochemistry to quantify the number and character of plaques in the frontal cortex of aged monkeys (*48*). Immature plaques were rich in AChE activity but contained little amyloid. Mature plaques showed marked AChE activity and substantial amounts of amyloid. End-stage plaques contained large amounts of amyloid but were devoid of neurites and showed little AChE activity. This investigation suggested that AChE-rich dystrophic neurites, presumably derived from nbM axons, were an early component of the plaque and that loss of these neurites was associated with the formation of burned-out plaques and reduction in AChE activity in the cortex (*48*). One can imagine that this process occurring repeatedly and affecting many cholinergic axons would result in multiple neuritic plaques and profound reductions in AChE and CAT activity in the cerebral cortex (*49, 50*). The fact that plaque density in cortex correlates both with reductions in CAT activity and with the severity of cognitive impairments in AD lends support to this hypothesis (*20*).

Specificity of the Nucleus Basalis Lesion to Alzheimer's Disease

The reductions in the presynaptic markers for the cholinergic neurons in the cerebral cortex and hippocampus in AD appear to be specific rather than reflecting a more global change in neuronal markers. Neurons that use GABA as their neurotransmitter are thought to be intrinsic to the cerebral cortex (*51*); for example, their cell bodies and axons are restricted to the cerebral cortex. The concentration of GABA and the activity of GAD, the enzyme that synthesizes GABA, have not been found to be consistently reduced in the cerebral cortex and hippocampus in AD (*21, 52*). Similarly, cholecystokinin (CCK), arginine-vasopressin, and vasoactive intestinal polypeptide (VIP), neuropeptides that are localized in neuronal cell bodies within the cortex, were not significantly decreased in AD (*22, 53–54*). Thus, markers for several neuronal systems intrinsic to the cerebral cortex exhibit neither the consistent nor the profound reductions demonstrated for the cholinergic systems.

However, it should be noted that concentrations of somatostatin, a neuropeptide believed to be localized in cortical bipolar neurons (*55*), may be significantly decreased in AD (*22*). Because of the growing evidence of the co-localization of neuropeptides in neurons that utilize the amine neurotransmitters (*56*), it seemed possible that the reduction of somatostatin reflected its partial localization in the affected cortical cholinergic fibers derived from the nbM. However, excitotoxin-induced lesions of the rat VGP did not decrease somatostatin levels in the cortex or hippocampal formation whereas excitotoxin lesions of the hippocampus caused a profound reduction in the somatostatin level without affecting CAT activity (*57*). Thus, the reduction in somatostatin levels in the cortex and hippocampus in AD appears to reflect alterations in another neuronal system, probably intrinsic to these regions and distinct from the nbM cholinergic pathways.

Several studies have been performed on the levels of the presynaptic markers for the noradrenergic neurons in the cerebral cortices of patients who died of AD; and the results have ranged from normal values to significant decrements (*19, 58, 59*). Degeneration of the locus coeruleus, the source of cortical noradrenergic innervation, has been reported to occur in younger patients suffering from AD, whereas this noradrenergic nucleus remains relatively intact in older patients who die with the senile form of the disorder (*60*). This observation points to a possible difference between the early and late age of onset of AD in



AcCh = Acetylcholine
CAT = Choline Acetyltransferase
AcEs = Acetylcholine Esterase

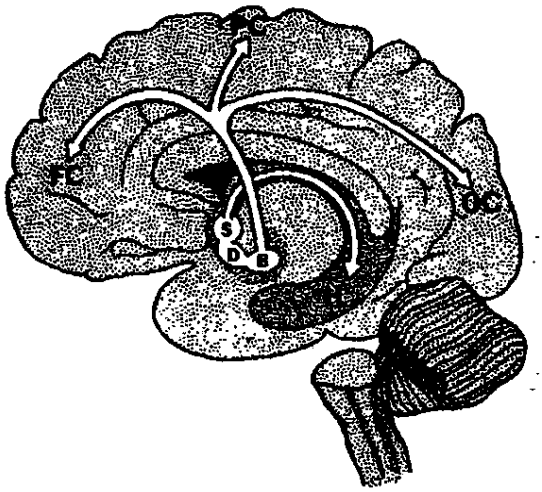


Fig. 2 (left). Schematic representation of a cholinergic synapse. Acetylcholine (*AcCh*) is synthesized from choline and acetyl-coenzyme A (*Ac-CoA*) by the enzyme choline acetyltransferase (*CAT*). Acetylcholine is stored within vesicles in the nerve terminal and released into the synaptic cleft upon depolarization. Acetylcholine diffuses across the cleft to activate muscarinic receptors, the predominant receptor in brain, or nicotinic receptors. The action of acetylcholine is rapidly terminated by hydrolysis by the enzyme acetylcholinesterase (*AcEs*) which is located on the surface of cholinergic neurons as well as neurons receiving cholinergic innervation. Fig. 3 (right). Cholinergic pathways innervating cortex. The cholinergic neuronal cell bodies of the basal forebrain located in the nucleus basalis of Meynert (*B*), the diagonal band of Broca (*D*), and the medial septal nucleus (*S*) send axons that innervate the entire cortex including the frontal (*FC*), parietal (*PC*), and occipital (*OC*) cortex, as well as the hippocampal formation (*H*).

spite of the similarities in the histopathology and cholinergic deficits. Thus, there is compelling evidence of degeneration of other neuronal systems, in addition to the basal forebrain cholinergic projections in AD, which most likely ntribute to the symptomatic manifesions of the disorder in, as yet, poorly defined ways.

In a related question, it is important to determine whether the degeneration of dbB and nbM neurons is specific for AD or occurs in other neuropsychiatric disorders. Parkinson's disease (PD), a disorder characterized by degeneration of the nigrostriatal dopaminergic pathway (*12*), is often complicated by a progressive dementia (*61*); and many of these patients show pathological changes (plaques and tangles) of AD (*62*). Nearly 20 years ago, Hassler (*63*) described loss of cells in the nbM in some cases of PD and implicated this lesion in what he termed "bradyphrenia," for example, a slowness of thought, delay in emotional reactions, and difficulty in decision-making. Similar changes were seen in some individuals over 70 years of age. Dementia in PD and lesions in the nbM have not been causally linked, however, until recent quantitative studies of nerve cells in the dbB and nbM have indicated that demented PD patients do have a selec- loss of this population of neurons, reas those PD patients with normal cognitive function do not show damage to the nbM (*64*).

Individuals with Down's syndrome, trisomy 21, often experience a progressive deterioration in their limited cognitive abilities beginning at approximately 30 to 35 years of age (*65*). Brains of affected patients show neuropathological changes virtually identical to those in AD (*66*). Moreover, the activity of CAT is reduced in the cortex and basal forebrain (*67*); and in at least one case, neurons were decreased in the nbM (*49*). Therefore, degeneration of cholinergic neurons in the dbB and nbM is not restricted to AD but occurs in other diseases characterized by deterioration of higher cognitive functions and the histopathological stigmata of AD. In contrast, Huntington's disease (HD) is an autosomal dominant disorder in which there is a profound but relatively selective degeneration of neurons located in the cerebral cortex and striatum, a structure in proximity to the nbM. Although a progressive dementia is a con- nt feature of HD, its symptoms dif- rom those of AD in that apraxias and aphasias generally do not occur (*68*). Notably, neurochemical and histopathological studies do not show neuritic plaques, reduction in the activity of CAT in the cerebral cortex (*13*), or alterations in the population of neurons in the basal nucleus (*69*).

Role of the Nucleus Basalis Lesion in Behavior

The role of the septal-dbB-nbM system in behavior and cognition is poorly defined. As noted above, studies in both man and experimental animals indicate that centrally active drugs, which block muscarinic cholinergic receptors, can cause a relatively selective disruption of recent memory and, at high doses, produce more global impairments of cognitive functions resulting in delirium (*14*). Considerable evidence points to an important role of the hippocampal formation in memory; and lesions of the medial septum or the fimbria, which transect the cholinergic pathways to the hippocampal formation, severely impair learning and recent memory in experimental animals (*70*). Thus, degeneration of neurons in the medial septum and dbB, which project to the hippocampus, may account for the impairments in recent memory that characterize the initial phase of AD.

Thus far, there seem to be no published studies on the behavioral consequences in experimental animals that results from a discrete lesion of the nbM. Studies of the activity of nbM neurons in the primate indicate that many cells exhibit rapid changes in firing in relation to different stimuli and behaviors (*71*). Some neurons in particular respond to the sight of food or to delivery of a food reward, suggesting a role of this structure in feeding behavior. More generally these nuclei may contribute to reward, learning, and attentional mechanisms. In the light of widespread projections from the nbM to all areas of the cerebral cortex, as well as to limbic structures including the amygdala, there is reason to speculate that loss of these cholinergic pathways may account for some of the emotional and cognitive difficulties that develop in the course of AD. The damage to intrinsic cortical neurons or the abnormalities in somatostatin immunoreactivity in AD have not been correlated with clinical abnormalities and are problems for future investigation.

Future Research in Alzheimer's Dementia

The delineation of cholinergic deficits in AD along with the selective degeneration of nerve cells in the dbB and nbM provides the first example of a major disorder of higher cognitive functions in which transmitter-defined neuronal pathways responsible for dementia have been identified. Moreover, this nerve cell population appears to be implicated in two other types of dementia, PD (*64*) and trisomy 21 (*67*), which are associated with AD-type pathology. These findings do not mean that cholinergic projections from neurons in the dbB and nbM are the only systems affected in these types of dementia. However, evidence available to date suggests that this transmitter system is involved most consistently and most severely in AD. The identified cholinergic lesion in AD has important implications for its diagnosis, treatment, and ultimately its prevention.

With regard to diagnosis, we need to know more about the relation between the cholinergic deficits, degeneration of component neurons in the dbB and nbM system, and the variation in the symptomatic manifestations of AD. The degenerative process may result in the appearance of neuronal-specific markers in the cerebrospinal fluid or blood or the development of specific immune responses that could serve as direct diagnostic tests for the disorder. With the development of positron emission tomography (*72*), it seems likely that in the future noninvasive probes of central cholinergic function may be developed that will allow for the assessment of cholinergic neuronal integrity in demented patients with the presumptive diagnosis of AD.

With regard to pathophysiology and treatment, it is interesting to compare AD and PD since both disorders appear to result primarily from the loss of a relatively small population of transmitter-specific neurons with cell bodies located in the base of the brain. In PD, the dopamine utilizing neurons of the substantia nigra, which innervate the striatum, degenerate (*12*). Considerable success has been achieved in reducing the symptoms of PD by treatment with the precursor of dopamine, L-dopa, to correct the deficiency in this neurotransmitter. Several groups have undertaken clinical studies to determine whether treatment with drugs that directly or indirectly potentiate cholinergic neurotransmission might be of therapeutic value for patients with AD. Some of the pharmacologic strategies explored in AD include administration of the precursors for ACh, choline, or lecithin (*73*), treatment with inhibitors of AChE to prolong the synaptic action of ACh (*74*), or treatment with drugs that directly stimulate the postsynaptic muscarinic receptor

(*75*). Thus far, the results have been rather inconclusive although a few reports indicate that some patients, primarily in the early stages of AD, may experience modest improvements in cognitive functions (for review, see *76*).

One possible explanation for relative lack of response to pharmacologic treatment in AD as compared to PD may lie in important differences in the synaptic organization and physiology of the two affected neuronal systems. Whereas the dopaminergic innervation of the striatum appears to be highly arborized and overlapping (*75–77*), the cholinergic fibers innervating the cortex exhibit a topographic, radial distribution (*42*, *43*). More importantly, the firing rates of dopamine neurons are very low and show no correlation with specific aspects of movement or behavior, suggesting a more general, modulatory influence on the striatum (*77*, *78*). In contrast, nbM neurons discharge at higher rates and are phasically active with rapid alterations in firing in relation to behavior (*71*). The loss of a neuronal system that exerts tonic modulatory influences, like the dopaminergic system, may be more amenable to pharmacologic correction than the loss of a neuronal system, like the nbM, which conveys spatially and temporally coded information. Successful approaches toward the treatment of AD, if feasible, will depend on a better delineation of the synaptic organization, physiology, and function of the cortical cholinergic projections. Animal models with selective lesions of the basal forebrain cholinergic complex may prove to be useful for preclinical testing of potentially therapeutic agents (*79*).

The identification of the neuronal systems affected in AD now allows neuroscientists to pose questions concerning mechanisms responsible for the selective degeneration. It is important to determine what features the cholinergic, noradrenergic, somatostatin-containing neurons, and possibly other affected neuronal populations share in common that render them vulnerable to the degenerative process. In other words, is it their size, extent of axonal arbor, type of afferent input or metabolic specialization? The interaction between age and the expression of selective vulnerability remains a critical issue that has relevance not only to AD but other hereditary neurodegenerative disorders such as HD. Insights into the molecular biological mechanisms involved in selective neuronal vulnerability may ultimately lead to the development of treatments that relieve or prevent the degenerative process of AD.

Summary

Alzheimer's disease and senile dementia of the Alzheimer's type, at present distinguished by age of onset, are characterized by progressive abnormalities of memory, behavior, and cognition. The brains of these patients show neurofibrillary tangles, neuritic plaques, and loss of specific populations of nerve cells. Neurochemical studies indicate that presynaptic cholinergic markers are markedly reduced in the cerebral cortex and hippocampus of affected individuals. This cholinergic deficiency appears to be due to a loss of neurons in the medial septum, diagonal band of Broca, and the nucleus basalis of Meynert, a basal forebrain cholinergic system which projects directly to the hippocampus and neocortex. Loss of this cell population also has been implicated in other types of dementia showing features in common with Alzheimer's dementia. The identification of a transmitter-specific pathway selectively affected in a major form of dementia is an important step in the design of diagnostic studies, investigations of pathogenic mechanisms, and the development of therapeutic approaches to these debilitating neuropsychiatric disorders.

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The Electric Power Research Institute

Chauncey Starr

Growing awareness of research needs, coupled with the threat of federal intervention, galvanized the leaders of the electric utility industry in the early 1970's. By January 1973, the Electric Power Research Institute (EPRI) was in business—in spite of skepticism from inside and outside the industry.

As EPRI's first president, I began with a budget of $61 million pledged by the industry for 1973 and imputations from many quarters that EPRI was a sham, that the utility industry was not serious about its technical responsibilities, and that this new entity would not get anywhere. This early history of the institute and of the legislation proposed by the Senate Commerce Committee as a result of the 1965 blackout in the Northeast have been recounted elsewhere (*1*).

National investments in research and development (R & D) are indirectly provided by the public through taxation, cost of goods, or direct contribution. EPRI is supported through the cost of electricity and represents a novel form of institutional intermediary between the consuming public, the utilities, and the researchers. Because many scientists in fields outside of energy research have had little contact with EPRI, I shall describe its scope, organization, and philosophy.

Dr. Starr is vice chairman and founding president of the Electric Power Research Institute, Palo Alto, California 94303.

Organization

EPRI is a nonprofit organization whose purpose is to manage a coordinated national R & D program for the electric power industry. EPRI selects and funds research projects designed to develop or improve technologies that will help the utility industry meet present and future electric energy needs in environmentally and economically acceptable ways. EPRI's activities are coordinated with those of government agencies, individual utilities, manufacturers and vendors, and comparable organizations in many other countries.

Of the roughly 3000 electric utilities in the United States, almost all the largest are voluntary supporting members of EPRI. In 1982, the 571 members were 160 investor-owned utilities, including their affiliates and service organizations; 177 municipal or regional government utilities; 232 rural electric cooperatives; and two federal systems—Tennessee Valley Authority and the Bonneville Power Administration. About 150 nonmember utilities also contributed some measure of support. Collectively, the contributors represent about 70 percent of the total electricity generated in the United States. EPRI also has 16 foreign utility associates with which information is exchanged.

In 1982, members paid 0.0236 cents per kilowatt-hour of electricity sold in 1980, or about 0.3 percent of member utilities' gross revenue, of which EPRI manages 80 percent and utilities retain and manage 20 percent for specific R & D needs. EPRI had a total budget of about $300 million in 1982, of which $260 million covered external R & D contract activities. Aside from membership payments from the Tennessee Valley Authority and Bonneville Power Administration, no federal funds come to EPRI, although many joint programs with federal agencies have been undertaken.

EPRI's primary areas of research are organized into six technical divisions (Fig. 1).

Since its founding, EPRI has initiated more than 1800 research projects (*2*). There are currently about 1400 active R & D projects under EPRI management. The 5-year funding plan (1982 to 1986) for these projects totals $1.8 billion. Cofunding and cost-sharing by contractors and other organizations increase

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Research report

Brain levels and acetylcholinesterase inhibition with galantamine and donepezil in rats, mice, and rabbits

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Abstract

Galantamine is a rather weak acetylcholinesterase (AChE) inhibitor, currently approved for the symptomatic treatment of Alzheimer's disease, with possible additional allosteric potentiating effects at the nicotinic ACh receptor (nAChR). Earlier data from in vitro biochemical tests suggest that donepezil is 40- to 500-fold more potent than galantamine in inhibiting AChE. In this study, both brain levels and K_i values for AChE inhibition for donepezil and galantamine in rat, mouse, and rabbit after subcutaneous application were determined. Clearance of galantamine from the brain is in general faster that donepezil and is faster in rabbits compared to rats and mice. The brain-to-plasma ratio for galantamine and donepezil, respectively, ranges from 1.2 to 1.5 in the rabbit, 3.3 to 5.2 in the mouse, and 6.6 to 13 in the rat. Galantamine doses between 1.5 and 5 mg/kg are appropriate to reach brain levels within the documented optimal allosteric potentiating ligand dose-response. K_i values of brain AChE inhibition for galantamine and donepezil, respectively, are 7.1 and 2.3 μg/g in rats, 8.3 and 0.65 μg/g for mice, and 19.1 and 1.3 μg/g in rabbits. The data also suggest that for a similar degree of brain AChE inhibition, 3–15 times higher galantamine than donepezil doses are needed.

Theme: Neurotransmitters, modulators, transporters and receptors
Topic: Acetylcholine

Keywords. AChE inhibitors; Rodent; Pharmacodynamics; Brain AChE; Pharmacokinetics

1. Introduction

Galantamine is a modest acetylcholinesterase (AChE) inhibitor, currently approved for the symptomatic treatment of Alzheimer's disease, with possible additional allosteric potentiating effects at the nicotinic ACh receptor (nAChR) (for a review, see. [28]. As both actions work in the same direction in terms of enhancing nicotinic cholinergic transmission, it is very difficult to identify the relative contributions of either process in an in vivo situation. Despite this, it is clear that for an optimal synergism between these two properties the local brain concentration of galantamine should fall within the optimal dose range reported for the allosteric potentiating ligand effect [18]. At the same time, it is of interest to know the degree of AChE inhibition by galantamine within this concentration range in order to allow a comparison to be made with pure AChE inhibitors in pre-clinical in vivo models employed in Alzheimer's disease research. Such an experimental strategy can start to address the in vivo significance of the nicotinic properties of galantamine relative to its AChE inhibitory

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properties in terms of the total behavioral or neurochemical outcome.

The affinity of galantamine for the AChE enzyme is small, the IC_{50} ranging from 800 nM in vitro and >2 μM in dog skeletal muscle [13] to values of 2 μM in rat cerebral cortex [24] and 2.4 μM ex vivo in human brain tissue [26]. In this last study, the inhibition of AChE by galantamine was similar in postmortem brain and brain cortical biopsies from patients submitted to brain tumor removal, indicating that postmortem changes up to 28 h after death were unlikely to influence the measurement of AChE inhibition. An important point to note is that in contrast to brain enzyme the IC_{50} of galantamine on the erythrocyte enzyme is much lower—about 365 nM [26]. This has been a potential source of confusion as to the potency of galantamine in the past.

As this study mainly deals with determination of substrate concentration independent K_i values for galantamine and donepezil, it is worthwhile looking at studies which have reported K_i values rather than IC_{50} values. For galantamine, values range from 120 nM in electrical eel tissue, over 200 nM in human erythrocytes [14], to 520 nM in recombinant human AChE [8].

In contrast to galantamine, the in vitro potency of donepezil for AChE inhibition is much higher. In human erythrocytes, donepezil has an AChE IC_{50} value of 6.7 nM [17], and in rat cerebral cortex an IC_{50} value of 13.5 nM [24]. In studies using recombinant human AChE [5,8], donepezil had K_i values of 24 and 1.1 nM, respectively.

The optimal brain levels for galantamine's allosteric potentiating ligand (APL) effect are based on the documented in vitro dose dependency of this effect in HEK293 cells expressing the human receptor [18] and the subsequent confirmations in rat hippocampal slices [19] and human neuroblastoma cells [7]. In all of these studies, the effective concentration range is between 100 nM and 1 μM. Given the molecular weight of galantamine, this translates into a concentration range of 35–350 ng/ml.

Nicotinic receptor stimulation by an allosteric potentiating ligand is an interesting therapeutic approach in Alzheimer's disease [4], and preclinical and clinical experience with galantamine in this context could help us understand the relevance of this approach in Alzheimer's disease.

The aim of this study was to define a relationship between dosage and brain levels so as to identify an appropriate in vivo dose range for galantamine in rats, mice, and rabbits in order to achieve galantamine brain levels in the optimal range for the APL effect. As the molecular weight of donepezil (415.96) is similar to the molecular weight of galantamine (368.2), the same doses of both compounds are almost equivalent to equimolar doses. At the same time, measuring the corresponding brain AChE inhibition level provides information for a comparison of galantamine with the effects of a pure AChE inhibitor, such as donepezil.

2. Materials and methods

2.1. Animal species

Mice (C57/BL6, male of 7 weeks of age, 23 g), rats (Sprague–Dawley rats, male, 6 weeks old, weighing 200 g), and rabbits (KBL New Zealand White, male, 18–20 weeks old, weighing 3.0 kg) Specific Pathogen Free (SPF) were obtained from Charles River Laboratories (l'Arbresle, France) and CEGAV s.sc (Saint-Mars-D'Egrenne, France). The study was conducted under protocols approved by the local Ethical Committee at the Centre International de Toxicologie, according to international guidelines of animal welfare. All together, 150 animals of each species were used.

2.2. Materials

Galantamine hydrobromide, donepezil hydrochloride, and $^{13}C^{2}H_3$-Galantamine hydrobromide were supplied by Janssen Research Foundation, now Johnson and Johnson Pharmaceutical Research and Development (a division of Janssen Pharmaceutica NV), Belgium. Benzetimide (Lot Number 76H0176) was supplied by Sigma-Aldrich. For AChE activity determination, the Roche kit was used (see below).

2.3. Preparation of animals

All animals were treated subcutaneously (sc) with an appropriate dose of galantamine or donepezil (0.05, 0.15, 0.5, 1.5, or 5 mg/kg) and three animals of each group were submitted at specific time points (0.25, 0.5, 1, 3, and 6 h after sc injection) to the following procedure. Subcutaneous application was chosen because of availability of toxicity data and widespread use in pharmacology research. Blood (1 mL) was collected from the heart under isoflurane anesthesia in EDTA tubes. Immediately after blood sampling, brains were perfused free of blood under isoflurane anesthesia, keeping the perfusion time as short as possible to minimize washout of drug (<1 min). Briefly, animals were placed on their back on an anesthetic induction board, and while monitoring the anesthesia, a ventral cutaneous incision was made in the neck region so as to expose the jugular vein. The thoracic cavity was opened, the descending aorta was clamped in the thoracic region, a cannula (0.8 mm × 19 mm) was inserted into the left cardiac ventricle, and 10–20 mL (for mice and rats) or 120 ml (for rabbits) 0.9% NaCl sterile solution injected in less than 1 min (the time and volume was standardized as much as possible for each animal). The jugular vein outflow was monitored until a clear perfusion fluid without traces of blood was obtained, indicating that the blood had been flushed out of the cerebral circulation. The brain was then rapidly removed, cut in two hemispheres, the weight of each hemisphere measured before snap-freezing in liquid nitrogen.

2.4. Preparation of samples

Blood was centrifuged to obtain plasma, which was stored at −80 °C until levels of galantamine or donepezil were measured.

Rat and mouse right brain hemispheres were transferred to labeled 10 ml screw-capped, round bottomed glass tubes. 5 ml of purified water was added to each tube which was subsequently vortex mixed for approximately 15 s then sonicated for 10 min. Methanol (1 ml) was then added and the tube again vortex mixed for approximately 5 s then rotary mixed for 30 min. Rabbit right brain hemispheres were transferred to labeled 60 ml screw-capped plastic tubes. 10 ml of purified water was added to each tube and the brains homogenized using a Silverson Homogeniser. Following homogenization, 2 ml of methanol was added to each tube and the homogenate vigorously vortex mixed. All tubes were then stored frozen at approximately −80 °C until analysis of galantamine or donepezil levels.

Left brain hemispheres obtained at 1 h post-treatment were kept on ice, dissected, and homogenized in TRIS–HCl buffer (10 mL of buffer per gram of brain) with a Potter mixer. Samples were centrifuged at +4 °C at 2500 rpm for 10 min, decanted, and 0.2 mL aliquots were stored at −80 °C until analysis of AChE activity.

2.5. Analysis of plasma and brain galantamine and donepezil levels

Analysis of plasma and brain drug levels was performed using LC-MS/MS, following a protocol developed at Johnson and Johnson Pharmaceutical Research and Development for galantamine and a method published for donepezil [15]. Briefly, between 8 and 11 mg of each compound was accurately weighed into a 10 ml volumetric flask and dissolved in methanol to give stock solutions between 650 and 990 μg/ml. Galantamine and donepezil were combined by transferring appropriate volumes of the stock solutions into a 10 ml volumetric flask and diluting with methanol/water (15: 85) to give a working stock solution of 10 μg/ml. This was then further diluted to give appropriate calibration and Quality Control (QC) spiking solutions covering a range of calibration concentrations.

$^{13}C^{2}H_{3}$-Galantamine and benzetimide were combined by transferring appropriate volumes of the stock solutions into a 10 ml volumetric flask and diluting with methanol/water (15: 85) to give a working stock solution of 1 μg/ml. An aliquot (100 μl) of the working stock solution (1 μg/ml) was transferred to a 10 ml volumetric flask and diluted with methanol/water (15: 85) to give a working solution of 10 ng/ml.

Depending on the calibration range and matrix (plasma or brain homogenate) used, samples were prepared in the following manner. A suitable aliquot of sample or control matrix was accurately pipetted into a 4 ml polypropylene tube and spiked with combined internal standard solution (100 μl; 10 ng/ml). For calibration and QC samples, a suitable volume of the appropriate combined calibration and QC spiking solution was also added. All samples were then diluted to a final volume of 1100 μl with purified water and briefly vortex mixed.

Vacuum was applied to all samples using the same automated solid phase extraction (SPE) method. Sample extraction was carried out on a Tecan Genesis using an Oasis HLB (1 ml, 30 mg; Waters) 96-well extraction block and between each liquid application vacuum is applied through an extraction manifold to draw the liquid through the extraction block. The SPE matrix was activated with 1 ml of methanol then equilibrated with 1 ml of purified water prior to sample application. An aliquot (1 ml) of the samples was transferred to the extraction block which was subsequently washed with 1 ml of methanol: purified water (5:95). The retained components were then eluted from the extraction block with 1 ml of methanol which was collected in a clean polypropylene 96-well collection block.

The methanol eluate was blown to dryness under a stream of oxygen-free nitrogen, the extracts reconstituted in 140 μl of methanol/purified water (15:85), and the collection block heat-sealed with a foil seal.

An analytical batch of samples comprised of 75 study samples (15 samples from each of 5 dose groups) along with calibration samples (at 8 different concentration levels over the calibration range) and quality control samples, in triplicate, at three different concentrations across the concentration range and spread throughout the study samples. For the repeat analysis of samples where batch sizes were smaller than 26, quality control samples were analyzed in duplicate.

All samples were analyzed by LC-MS/MS using an HPLC system comprising Perkin-Elmer Series 200 Micro pumps and a Perkin-Elmer Series 200 Autosampler (Perkin-Elmer, Beaconsfield, UK) coupled to an Applied Biosystems API 3000 triple quadrupole mass spectrometer (Applied Biosystems, Warrington, Cheshire, UK) fitted with a Turbo IonSpray (TIS) source. A Valco 2-position switching valve was also used as a divert valve. Chromatography was carried out on an ACE aqueous 5 μ column (50 × 3.0 mm; Hichrom, Reading UK). The sample aliquot injected onto the LC-MS/MS system was 100 μl with an autosampler needle wash of methanol/purified water (50:50). A gradient HPLC system was used with mobile phases of (A) aqueous, formic acid (0.2%) and (B) methanol using the gradient at a total flow rate of 1 ml/min.

Data were acquired using proprietary software from the instrument manufacturer; Sample Control V1.4 (Applied Biosystems, Warrington, Cheshire, UK). Peak area and quantification data were generated by MacQuan software Version 1.6 (Applied Biosystems, Warrington, Cheshire, UK).

Inversely weighted ($1/x^2$) linear regression analysis was used to construct calibration curves and the test sample concentrations were determined by interpolation of the peak area ratios into the calibration curve equation.

Table 1
Area under the curve (AUC) in ng h/ml for the different conditions studied

Species	Dose (mg/kg)	Brain AUC (ng h/g)		Plasma AUC (ng h/ml)	
		Galantamine	Donepezil	Galantamine	Donepezil
Mouse	0.05	90	37	13	4
	0.15	122	73	62	17
	0.5	363	565	154	68
	1.5	2506	537	583	201
	5	3203	1344	2772	862
Rat	0.05	23	15	15	9
	0.15	350	43	47	25
	0.5	1712	1601	144	69
	1.5	4206	8415	456	262
	5	4028	7213	1466	982
Rabbit	0.05	18	36	15	20
	0 15	50	121	42	74
	0.5	157	283	125	181
	1.5	563	943	661	636
	5	2328	2841	1437	2823

This is based upon three animals per dosage and per time point (every figure is the result of studies in 15 animals).

2.6. Brain AChE determinations

AChE levels were determined [9] with a Hitachi 717 automate using the Roche/Hitachi 717 method at 37 °C as described below. Briefly, chromogenic buffer reagent R1 (50 mmol/L of phosphate buffer, pH 7.2, containing 0.25 mmol/L of dithiobisnitrobenzoic acid) together with variable concentrations of reagent R2 (acetylthiocholine (0.03, 0.05, 1 or 0.5 mmol/) was added to 3 μL of brain homogenate. A total of 353 μL (300 μL of reagent R1 and 50 μL of reagent R2) was sampled by the Hitachi apparatus. Optical density (OD) was measured at 480 and 660 nm, for a total of 10 min (first after adding the reagent R1 for 5 min and then after adding the reagent R2 for an additional 5 min). Since the coloration measured is proportional to the AChE activity, the slope of the observed OD changes is proportional to the speed of the hydrolysis reaction, which is then used in the calculation of the K_i values, using Lineweaver–Burk plot methods.

2.7. Data analysis plasma and brain galantumine and donepezil

Plasma or brain concentrations were averaged per time point, dose level, compound, and species. Plasma and brain area-under-the-curve until the last detectable concentration (AUC) values were calculated according to the linear logarithmic trapezoidal rule [10] per dose level, compound, and species.

2.8. Data analysis brain AChE activities

The calculation of K_i values [27] for galantamine and donepezil gives values essentially independent of the substrate concentration used. The hydrolysis velocity of 4 different concentrations of acetylthiocholine substrate was obtained and a $1/V$ vs. $1/S$ relationship was calculated and plotted using Lineweaver–Burk methods. Using appropriate equations for competitive and non-competitive inhibition, the K_i values for donepezil and galantamine could be calculated.

3. Results

3.1. Brain levels of galantamine and donepezil

Plasma and brain levels of galantamine or donepezil were measured at five time points (0.25, 0.5, 1, 3, and 6 h) after five different doses of the compounds (0.05, 0.15, 0.5, 1.5, and 5 mg/kg). The brain AChE inhibition was only measured at the 1 h time point.

Table 1 shows the values for the area-under-the-curve (AUC) for all conditions in this experiment. Figs. 1, 2, and 3 show the time-dependent changes in brain and plasma concentration for donepezil and galantamine for the rat, mouse, and rabbit, respectively, determined for 3 animals per time point and per dosage. There are interesting differences among the three species studied. After subcutaneous administration of identical doses, galantamine plasma levels are higher than those of donepezil in the mouse and the rat and similar or lower in the rabbit. However, this difference is much less in the brain, suggesting that donepezil had a better brain penetration than galantamine. The donepezil values tend to decrease more slowly in the brain than the galantamine levels, suggesting a higher retention rate.

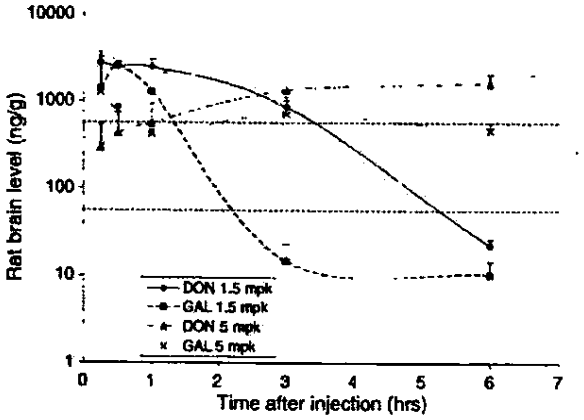

Fig 1. Time-dependent changes of galantamine and donepezil levels in rat brain (in ng/mg) after sc injection of 5 and 1.5 mg/kg, respectively. Average values and standard deviation are given on 3 animals per time point and per dosage. Note the logarithmic concentration scale. The dotted lines indicate the optimal window for the allosteric potentiating ligand effect for galantamine determined from in vitro studies. The figure suggests that this optimal level for APL effect is readily achieved for galantamine concentrations of 1.5–5 mg/kg at various time points. The peak values are reached very rapidly for donepezil (within the first 15 min) and somewhat slower than for galantamine (30 min). Under these conditions, for the same in vivo dosing, the brain uptake of donepezil is higher, while the clearance of brain donepezil is slower than that of galantamine.

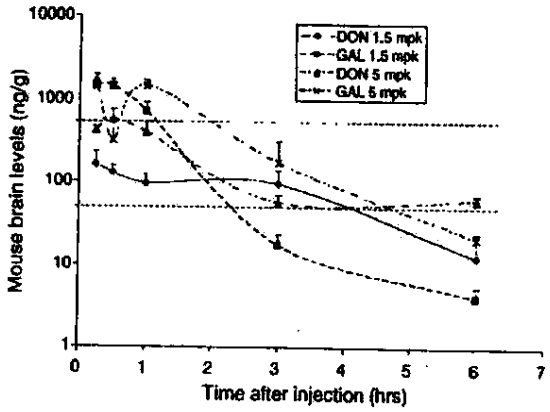

Fig. 2. Time-dependent changes of galantamine and donepezil levels in mouse brain (in ng/mg) after sc injection of 5 and 1.5 mg/kg, respectively. Average values and standard deviation are given on 3 animals per time point and per dosage. Note the logarithmic concentration scale. The dotted lines indicate the optimal window for the allosteric potentiating ligand for galantamine effect determined from in vitro studies.

The peak brain concentration is reached after a period between 15 (donepezil) and 30 min (galantamine) after sc administration. Galantamine is cleared out of the brain faster than donepezil especially in the rabbit.

The absolute area-under-the-curve (AUC) values of both donepezil and galantamine in plasma and brain were mostly linearly related to the dose with correlation coefficients consistently at 0.95 or above. There was a slight deviation from linearity in mouse brain donepezil exposure (correlation coefficient 0.89) and in rat brain galantamine and donepezil exposure (correlation coefficients 0.76 and 0.73, respectively).

The optimal window for the APL effect of galantamine is achieved for doses between 1.5 and 5 mg/kg at various time points.

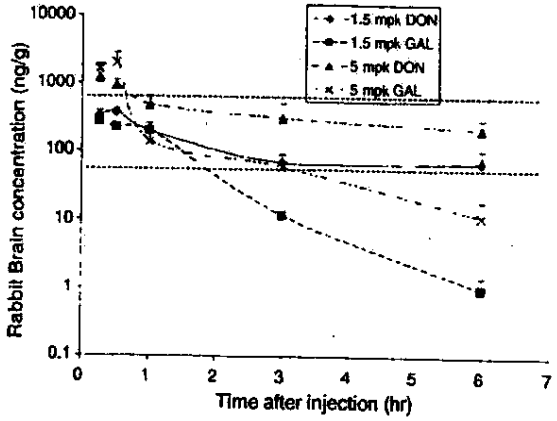

Fig. 3. Time-dependent changes of galantamine and donepezil levels in rabbit brain (in ng/mg) after sc injection of 5 and 1.5 mg/kg, respectively. Average values and standard deviation are given on 3 animals per time point and per dosage. Note the logarithmic concentration scale. The dotted lines indicate the optimal window for the allosteric potentiating ligand effect for galantamine determined from in vitro studies.

3.2. Brain acetylcholinesterase inhibition levels for donepezil and galantamine

The V_m and K_m values for acetylthiocholine hydrolysis in the different brain extracts (in the absence of AChE inhibitors) are very similar for the three species. In the rat, K_m is 52 μM, in the mice 56 μM, and in the rabbit 59 μM, generally in line with other findings [8].

For calculating the Lineweaver–Burk plot, the highest dosages were used to determine slope and intercept in the relation ($1/s_0$) vs. ([I]) for the competitive inhibitor galantamine and in the relation ($1/v_o$) vs. [I] for the non-competitive inhibitor donepezil (at least two doses and the control). At the lowest doses, both drugs were unable to provide a noticeable inhibition of the enzyme. The K_i values for donepezil and galantamine are shown in Table 2.

The ratio of relative potencies of AChE inhibition for donepezil vs. galantamine in blocking AChE ranges from 3.1 in the rat to 13 in the mouse and 15 in the rabbit in terms of brain concentrations. However, because the time-dependent absolute brain levels of galantamine and donepezil are different, the corresponding K_i values for sc dosages are different. In fact, when we analyze the Lineweaver–Burk plot at 1 h after application in terms of the subcutaneous doses, we find that the K_i value in the rats is 22.8 mg/kg for galantamine and 1.5 mg/kg for donepezil (a ratio of 15), 26.5 mg/kg for galantamine and 7.6 mg/kg for donepezil in mice (a ratio of 3.5), and 35.5 mg/kg for galantamine and 15.2 mg/kg for donepezil in the rabbit (a ratio of 2.3).

As we determined K_i values from the absolute brain concentrations, we can now calculate the instantaneous brain AChE inhibition as a function of time after sc injection using the experimentally determined brain levels for galantamine and donepezil at each time point. Assuming that the k_{off} values of both drugs are fast compared to the time-scale under investigation (minutes to hours), the inhibition I can be calculated as I = Conc / (Conc + K_i), where Conc is the brain level of galantamine and donepezil, respectively. For example, Fig. 4 shows the calculated brain AChE inhibition levels for galantamine and donepezil at different time-points after sc injection in the rabbit. It is clear that the amount of

Table 2
Calculated brain K_i values (in μg drug per gram brain weight) of brain AChE inhibition in the different species for the two drugs

Brain K_i	Galantamine (μg/g)	Donepezil (μg/g)
Rats	7.11	2.32
Mice	8.28	0.650
Rabbits	19.09	1.29

The determination is based upon a Lineweaver–Burke analysis starting from the average brain concentration measured in the first part of the study. Hydrolysis of the substrate at each concentration was measured in each of the three individual brains after 30 min (roughly the time of maximal brain levels) and this for all dosages (including the control). Based upon the graphical analysis of the Lineweaver–Burke plot, these inhibitions are generally of a non-competitive nature for donepezil and of a more mixed competitive–non-competitive nature for galantamine.

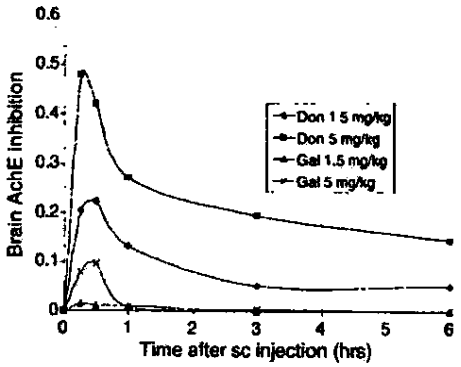

Fig. 4. Calculated brain AChE inhibition levels in the rabbit after sc injection of various drug doses using the formula I = Conc / (Conc + K_i), where K_i values in brain concentration are determined from Lineweaver–Burk analysis (see Table 2) and Conc are the experimentally observed mean brain levels of donepezil and galantamine. It is clear from this figure that at all dosages donepezil is four to ten times more potent in inhibiting the brain AChE.

inhibition in vivo by donepezil at all times is 4–8 times higher than for galantamine at the same dose. It is also clear from this figure that donepezil tends to stay much longer in the brain (probably longer than the 6 h we have probed here), while galantamine's activity is essentially gone after 3 h. Under these conditions of short-term treatment, very probably no or a very small upregulation of the AChE as a consequence of enzyme block is expected [21].

These data can now also be used to calculate actual brain AChE inhibition levels for any other administration protocol (i.e., iv, ip, or oral), provided that brain levels of the drugs are available.

4. Discussion

This is the first report directly comparing donepezil and galantamine, two drugs approved for mild-to-moderate AD, on brain drug levels and brain AChE inhibition in mice, rats, and rabbits. The purpose of this study was to estimate the degree of brain AChE inhibition in various experimental conditions where those drugs have been used. In addition, it also provides guidance to design and interpretation of acute experiments, as we can calculate a time-dependent estimate of the transient brain levels and AChE-inhibition activities after subcutaneous injection.

Brain levels of galantamine and donepezil increase transiently with a maximum between 15 and 30 min after sc injection. Clearance out of the brain was faster for galantamine than for donepezil. This is in line with both preclinical and clinical data [14,16]. These two studies also suggest that there is no accumulation of galantamine over longer time in rats and mice.

Interestingly, the plasma levels of both donepezil and galantamine in the rabbit are significantly lower than the plasma levels at corresponding times in the rat and mice, suggesting that the metabolism of the rabbit is faster than that of the rodents for both drugs.

The study also suggests that the optimal conditions–in terms of brain concentrations–for the allosteric potentiating ligand effect of galantamine can be achieved by doses ranging from 1.5 to 5 mg/kg in the three species studied.

The K_i values for galantamine and donepezil inhibition of brain AChE were determined under the same conditions as from which drug brain concentration was measured. This extends significantly the usefulness of this observation. As available evidence suggests that there is no time-dependent change in K_i values as a function of exposure time of the AChE enzyme to either drug [5,8], we can then extrapolate the observations to any other condition, provided brain levels are known. The ratios of K_i values in brain concentration between donepezil and galantamine range from 2.3 in the rat to 15 in the rabbit, much less than the 40- to 100-fold difference measured in vitro. This suggests that a large majority of donepezil is probably unable to access the cholinergic synapse and therefore unable to block the AChE. One possible explanation could be nonspecific binding to brain proteins, which could be in line with observations that galantamine has a low binding affinity to plasma proteins (18%) [14] compared to donepezil (98%) [22,25]. Alternatively, donepezil can be sequestered in a different compartment or galantamine can accumulate in the cholinergic synapse.

Based upon those data, relevant brain AChE inhibition levels for galantamine and donepezil after sc injection can be estimated, because we know the respective brain levels and this report suggests that pure brain levels are linear with the dose. This assumes that the window of exposure to the drugs is short enough not to induce upregulation of the AChE enzyme through alternative splicing [21]. For instance, 1 h after a sc injection of 3 mg/kg galantamine in rats, we would expect a 10% brain AChE inhibition, a 12% inhibition in mice, and a 1% inhibition in rabbits. In contrast, 1 h after a sc injection of 3 mg/kg donepezil, we would expect a maximal brain AChE inhibition of 39% in rats, a 29% inhibition in mice, and a 21% inhibition in rabbits. This strongly suggests that galantamine at in vivo concentrations of 3 mg/kg usually yields an acute brain AChE inhibition in the 1–12% range, whereas for similar in vivo doses for donepezil brain AChE inhibition is in the 20–40% range (i.e., 3- to 4-fold higher).

The levels of brain AChE inhibition by donepezil are similar to those observed [12] in young and aged rats, where 1 h after oral administration donepezil blocks the brain AChE by 40–70% in a dose range from 1.25 to 5 mg/kg. This study also suggests that the pharmacological AChE inhibition effects of donepezil in the brain are much more pronounced than in other tissues, in line with our observations that donepezil tends to be preferentially accumulated in the brain.

In a previous rat study comparing the effect of a 3-month chronic treatment with donepezil and galantamine, delivered

by Alzet minipumps [1], in vivo K_i values for brain AChE inhibition were calculated using a similar Lineweaver–Burk plot approach. The authors found a K_i of 0.19 mg/kg for galantamine and 0.45 mg/kg for donepezil. This suggests that donepezil is less potent in vivo in blocking the brain AChE level compared to galantamine and appears to be at odds with the results of this study. However, a re-analysis of the Lineweaver–Burk plot in Fig. 1 of the study by Barnes [1] actually suggests the opposite, e.g., that donepezil is the more potent AChE inhibitor. By applying a similar algorithm as described above to the data in found in Barnes [1], we find almost a four times higher K_i value for galantamine than for donepezil (2.99 mg/kg for galantamine vs. 0.75 mg/kg for donepezil), which is more in line with our experiments.

In previous studies on mouse forebrain, AChE was shown to be inhibited by 18% 1 h after 5 mg/kg sc and by 20% at 10 mg/kg galantamine [3]. This corresponds very well with our calculated inhibition levels of 15% and 25%, respectively, for 5 and 10 mg/kg galantamine. In mice, iv treatment with 8 mg/kg galantamine yielded about 43% inhibition of brain homogenate AChE and the brain-to-plasma ratio was 2.1, in line with our results [2].

The calculated levels of brain AChE inhibition for donepezil in this study are somewhat lower than in other reports. A recent study has found a 39% brain AChE inhibition in aged rats 18 h after the last bidaily ip injection of 1.5 mg/kg donepezil [20]. In this study, brain AChE was measured after chronic treatment of 21 days. This would be compatible with a K_i of 4 mg/kg (about half the value obtained in this paper). However, chronic twice a day injections combined with the slow donepezil brain clearance as observed in our study (see Fig. 1) is expected to have resulted in accumulation of donepezil in the rat brains compared to our single dose experiment. Oral donepezil in rats at doses of 1 and 2 mg/kg yielded less than 10% acute inhibition of the brain AChE [6].

The results of this present study allow a more detailed interpretation of previously published in vivo data sets in which donepezil and galantamine were compared. For example, in studies where similar doses of galantamine and donepezil have been used and an equivalent outcome has been recorded, e.g., the classical eyeblink conditioning study in rabbits [29] and the protection against cocaine and morphine induced stereotypical and hyperlocomotory effects in mice [23], our results suggest that the inhibition of the brain AChE is probably 3- to 4-fold higher in the donepezil-treated animals than in the galantamine-treated animals. Although actual brain ACh levels have not been measured in these experiments, it has been shown many times that higher AChE inhibition leads to higher free ACh levels in the cholinergic synapse. In addition, because galantamine's potency to inhibit Butyrylcholinesterase (BuChE) is much lower than inhibition of AChE [8,26], it is assumed that the contribution of galantamine's BuChE inhibition to free ACh levels is minimal. Therefore, in order to account for the same observed behavioral effects, an additional mechanism of action is needed to explain this equivalent behavior.

Taken together, these data suggest that many of the observed effects of the drugs in preclinical animal models arise at a relatively low level of brain AChE inhibition, both for donepezil (in the 20–30% range) and for galantamine (5–10%). This is in line with observations in Alzheimer patients receiving donepezil treatment, where in vivo brain AChE inhibition levels at clinically relevant doses have been determined to be in the range between 30% and 40% compared to untreated patients [11].

In summary, this study provides a reference for comparing the in vivo effects of galantamine vs. donepezil in animal studies. It permits comparison of conditions of equal dose for both drugs, where the brain AChE inhibition by donepezil is much higher than for galantamine. If in such studies both compounds have the same in vivo effect, this argues strongly for an additional mechanism of action for galantamine to make up for the difference in AChE inhibition. Allosteric modulation of nicotinic ACh receptors by galantamine is a prime candidate for this effect [28,30].

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EXHIBIT 26

Galantamine in AD

A 6-month randomized, placebo-controlled trial with a 6-month extension

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Article abstract—*Background:* Galantamine is a reversible, competitive cholinesterase inhibitor that also allosterically modulates nicotinic acetylcholine receptors. These mechanisms of action provided the rationale for a therapeutic trial of galantamine in AD. *Methods:* A 6-month, multicenter, double-blind trial was undertaken in 636 patients with mild to moderate AD. Patients were randomly assigned to placebo or galantamine and escalated to maintenance doses of 24 or 32 mg/d. Eligible patients then entered a 6-month, open-label study of the 24 mg/d dose. Primary efficacy measures were the 11-item AD Assessment Scale cognitive subscale (ADAS-cog/11) and the Clinician's Interview-Based Impression of Change plus Caregiver Input (CIBIC-plus). The Disability Assessment for Dementia (DAD) scale was a secondary efficacy variable. *Results:* Galantamine significantly improved cognitive function relative to placebo; the treatment effects were 3.9 points (lower dose) and 3.8 points (higher dose) on the ADAS-cog/11 scale at month 6 ($p < 0.001$ in both cases). Both doses of galantamine produced a better outcome on CIBIC-plus than placebo ($p < 0.05$). Therapeutic response to galantamine was not affected by *APOE* genotype. At 12 months, mean ADAS-cog/11 and DAD scores had not significantly changed from baseline for patients who received galantamine 24 mg/d throughout the 12 months. The most common adverse events, which were predominantly gastrointestinal, decreased in frequency during long-term treatment. There was no evidence of hepatotoxicity. *Conclusions:* Galantamine is effective and safe in AD. At 6 months, galantamine significantly improved cognition and global function. Moreover, cognitive and daily function were maintained for 12 months with the 24 mg/d dose. **Key words:** AD—Galantamine—Nicotinic receptors—Allosteric modulation—Acetylcholinesterase inhibition—Randomized controlled trial—Long-term efficacy—Tolerability.

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Impairment of cholinergic function in AD contributes to the cognitive deficits that are characteristic of the illness.[1] A deficit of central presynaptic cholinergic function has been demonstrated in AD, as indicated by decreased activity of choline acetyltransferase in the hippocampus and neocortex,[2] and by degeneration of cholinergic neurons in the basal forebrain.[3,4] These findings provide the rationale for cholinergic enhancement as an approach to improving cognitive function in AD.[5] Acetylcholinesterase (AChE) inhibitors represent one way of implementing this strategy. They inhibit the enzyme that hydrolyzes acetylcholine (ACh), thereby increasing its availability for synaptic transmission. AChE inhibitors are the only class of drugs that have consistently produced improvements in cognitive function relative to placebo, in studies of up to 6 months' duration.[6] However, their long-term efficacy has been questioned.[7]

Combining cholinergic pharmacologic mechanisms has been proposed as a strategy for enhancing clinical benefit in patients with AD.[5] Galantamine is a novel agent that reversibly and competitively inhibits AChE[8,9] and allosterically modulates nicotinic ACh receptors.[10,11] Galantamine's modulatory effect on nicotinic receptors potentiates the response of these receptors to ACh.[11] This enhancement of cholinergic nicotinic neurotransmission may be of clinical relevance because activation of presynaptic nicotinic receptors increases the release of ACh.[12-14]

We evaluated the efficacy and safety of two doses of galantamine compared with placebo over 6 months in patients with mild to moderate AD. Following this double-blind phase, the long-term effects of galantamine were monitored in patients eligible for an open-label extension phase of an additional 6 months.

See also page 2269

*See the Appendix on page 2267 for a listing of members of the Galantamine USA-1 Study Group.

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Methods. *Patients.* Inclusion criteria were 1) a history of cognitive decline that had been gradual in onset and progressive over a period of at least 6 months; 2) a diagnosis of probable AD according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer Disease and Related Disorders Association (NINCDS-ADRDA);[15] and 3) presence of mild to moderate dementia: a Mini-Mental State Examination (MMSE)[16] score of 11 to 24 *and* a score of ≥12 on the standard cognitive subscale of the AD Assessment Scale (ADAS-cog).[17]

Patients with stable and well-controlled concomitant medical disorders such as hypertension, heart failure (New York Heart Association class I or II), noninsulin-dependent diabetes mellitus, and hypothyroidism were included. Patients were excluded if they had evidence of any neurodegenerative disorders other than AD, cardiovascular disease thought likely to prevent completion of the study, clinically significant cerebrovascular disease, active major psychiatric disorders, hepatic, renal, pulmonary, metabolic or endocrine conditions or urinary outflow obstruction, an active peptic ulcer, or any history of epilepsy, drug abuse, or alcohol abuse.

Patients who had been treated for AD with a cholinesterase inhibitor in the preceding 3 months were also excluded. Any other antidementia medication had to be discontinued before entry to the study. The use of drugs for concomitant conditions was permitted during the study, except sedative—hypnotics and sedating cough and cold remedies, which were discontinued, if possible, 48 hours before cognitive evaluation. Any other drugs with anticholinergic or cholinomimetic effects were avoided where possible. Patients were eligible to enter the extension phase of the study if they had completed the first phase and still did not meet the initial exclusion criteria.

All eligible patients had a responsible caregiver, who, together with the patient (or appropriate representative), provided written informed consent to participate in the study. The study was conducted according to the Declaration of Helsinki and subsequent revisions, and approved by institutional review boards at each center or centrally.

Design. The initial phase of the study was a 6-month, parallel-group, placebo-controlled, double-blind trial undertaken at 33 sites in the United States. Following a 4-week, single-blind placebo run-in period, patients were randomly assigned (using a computer-generated code) to one of two oral galantamine treatment groups or placebo. Both the active treatment groups received galantamine 8 mg/d for the first week, followed by 16 mg/d in the second and 24 mg/d in the third week. In the fourth week, one group continued to receive the 24 mg/d dose; in the other, the dose was increased to 32 mg/d. Patients then continued with their target dose of galantamine or placebo for an additional 5 months. Galantamine and placebo were administered as identical single tablets taken twice daily.

All patients who entered the extension phase received open-label galantamine 8 mg/d in week 1, 16 mg/d in week 2, and then 24 mg/d for 5.5 months. At all doses, galantamine was administered as single tablets taken twice daily. Restrictions on the use of concomitant medications during the extension study were the same as during the double-blind phase. Throughout the 12 months, investigators remained blinded to the treatment to which patients had been randomly assigned at the start of the double-blind phase.

Outcome measures. Efficacy. The primary efficacy measures were the standard 11-item ADAS-cog subscale (ADAS-cog/11), with a score range of 0 to 70,[17] and the Clinician's Interview-Based Impression of Change plus Caregiver Input (CIBIC-plus).[18] The CIBIC-plus was scored by a trained clinician based on separate interviews with the patient and the caregiver. Scores ranged from 1 (markedly improved compared with baseline) to 7 (markedly worse).

Secondary efficacy variables included the expanded (13-item) version of the standard ADAS-cog subscale (ADAS-cog/13) with a score range of 0 to 85[19] and the proportions of "responders," defined as improvement in ADAS-cog/11 of ≥4 points compared with baseline.[20] Activities of daily living (ADL) were assessed with the Disability Assessment for Dementia (DAD) scale, which is based on interviews with the caregiver and assesses basic ADL, instrumental ADL, leisure activities, initiation, planning and organization, and effective performance; there are 46 questions, with a score range of 0 to 100.[21]

Assessments were performed at baseline, at 3 weeks (ADAS-cog only), and then at 3 and 6 months in the double-blind phase of the study. The ADAS-cog/11, CIBIC-plus, and DAD assessments were performed after 3 and 6 months in the extension phase. All efficacy variables were analyzed as a change from baseline (baseline referred to the first visit in the 6-month double-blind phase), except for CIBIC-plus at 12 months, which was analyzed as a change from the initial visit of the open-label phase.

Safety. Safety evaluations conducted throughout the study comprised physical examinations, EKG, vital sign measurements, and standard laboratory tests (blood chemistry, hematology, urinalysis). Monitoring for adverse events (classified according to World Health Organization Preferred Terms) were recorded weekly for the first month of both the double-blind and open-label phases of the study, and at monthly intervals thereafter.

APOE genotyping. A blood sample was taken at baseline to assess whether *APOE* genotypes affected response to galantamine. DNA was purified from whole blood samples according to standard protocols, and *APOE* genotyping was performed as previously described by Wenham et al.[22]

Statistical analysis. Data from an early phase II trial on galantamine (data on file, Janssen Research Foundation) indicated that about 125 patients were needed in each treatment group of the double-blind phase to achieve 80% power (α = 0.025 with a Bonferroni adjustment) to detect a difference of 2.75 points in the change in ADAS-cog/11 score between placebo and galantamine.

All randomly assigned patients who took at least one dose of trial medication were included in the analyses of baseline characteristics and safety data. The primary analysis of 6-month efficacy data were based on patients who also provided postbaseline data for any of the ADAS-cog/11, CIBIC-plus, or DAD variables at designated assessment times—a traditional "observed cases" (OC) analysis. Furthermore, to confirm the robustness of the efficacy results, a more conservative 6-month intention-to-treat (ITT) analysis was performed using the last-observation-carried-forward (LOCF) method (i.e., the last postbaseline observation available for each randomly assigned patient who

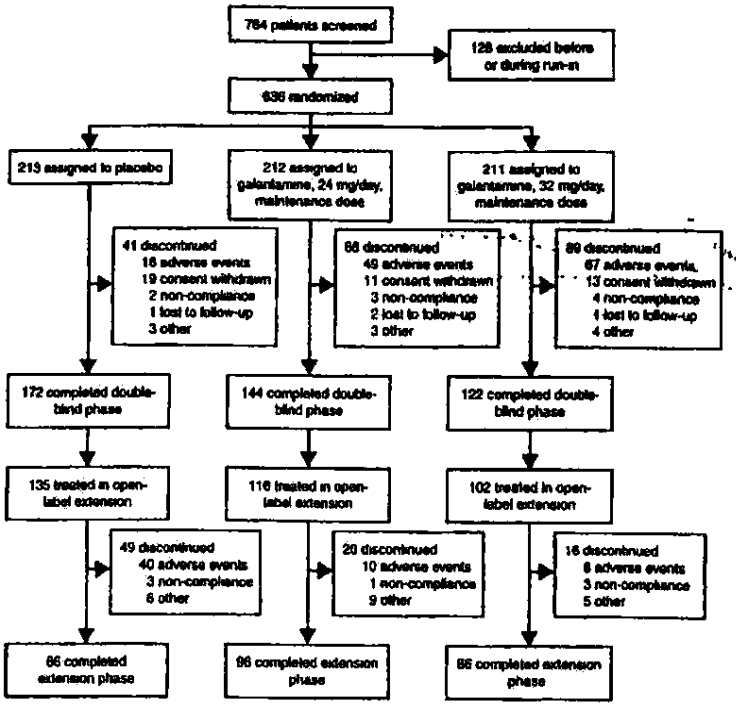

Figure 1. Trial profile.

received treatment). For the extension study, OC and ITT analyses were performed. All results discussed are based on OC analysis unless otherwise stated.

Baseline characteristics of the different treatment groups were compared using two-way analysis of variance (ANOVA) for continuous variables, and the generalized Cochran-Mantel-Haenszel test for categorical variables. Changes in outcome variables, vital signs and body weight from baseline were assessed using two-tailed, paired *t*-tests. Comparisons of variables between each galantamine group and the placebo group were made with the ANOVA model, including treatment and investigator as factors, and pairwise Dunnett's tests for changes from baseline in ADAS-cog subscales and DAD during the double-blind phase. An analysis of covariance (ANCOVA) model was also used in the analysis of change from baseline score, with baseline ADAS-cog value as a covariate. The ANCOVA and ANOVA models produced similar conclusions; therefore, results based on the ANOVA model are reported in this paper. Treatment by investigator interaction was tested and removed from the model as it was not significant at the 5% level. Generalized Cochran—Mantel—Haenszel tests were used to compare ADAS-cog/11 response rates and Van Elteren tests[33] for CIBIC-plus. ANOVA with pairwise Fisher's least-significant-difference tests were used to compare changes from baseline in vital signs, body weight in the double-blind phase, and ADAS-cog subscales and DAD during the extension phase. The time—response relationship for change in ADAS-cog/11 was analyzed using generalized linear interactive modelling, and exploratory ANOVA was used to investigate any relationship between baseline characteristics, including *APOE* genotype and changes in ADAS-cog/11. The statistical software used in these analyses was SAS Version 6.12 (SAS Institute, Cary, NC).

Results. Figure 1 illustrates the trial profile. Of the 764 patients screened for the initial phase, 636 were randomly assigned to trial medication, of whom 438 (69%) completed the double-blind phase. Of these 438 subjects, 353 entered the open-label extension, of whom 268 (76%) completed the study. The baseline demographic and medical characteristics of the three treatment groups were comparable (table 1). The only significant difference between the groups at baseline was the time since diagnosis of probable AD ($p = 0.02$), although the magnitude of this difference is unlikely to be clinically meaningful.

During the double-blind phase, the proportions of patients taking concomitant psychotropic medications, or taking them within 48 hours of the 6-month cognitive assessment, were similar in each group. On entry to the extension phase of the study, the baseline characteristics of the patients in each treatment group remained comparable.

As protocol deviations occurred in only 62 (10%) of randomly assigned patients (41 of whom used prohibited medication) and were comparable across the treatment groups, no per-protocol analyses were performed for the double-blind phase. Similarly, no per-protocol analyses were performed in the extension phase due to a low rate (12%) of protocol deviations.

Primary efficacy variables. At 6 months, OC analysis demonstrated a significant difference in the change in ADAS-cog/11 scores between galantamine- and placebo-treated patients (table 2). The differences in favor of galantamine were 3.9 points for the 24 mg/d and 3.8 points for

Table 1 Baseline characteristics

Characteristic	Placebo group (n = 213)	Galantamine 24 mg/d group (n = 212)	Galantamine 32 mg/d group (n = 211)
Men/women	82/131	73/139	87/124
Age, y*	75.3 ± 0.6	75.9 ± 0.5	75.0 ± 0.6
Weight, kg*	67.1 ± 1.0	67.6 ± 1.0	67.3 ± 1.0
White race, n (%)	196 (92.0)	195 (92.0)	190 (90.0)
Other active medical conditions, n (%)	203 (95.3)	200 (94.3)	194 (91.9)
≥1 *APOE*-ε4 allele, n (%)	113 (58.2)	120 (60.0)	116 (61.7)
Time since probable AD diagnosed, y*	1.13 ± 0.11	1.02 ± 0.10	1.45 ± 0.13
Total MMSE score*	19.2 ± 0.3	19.5 ± 0.3	19.1 ± 0.3
ADAS-cog/11 score*	25.7 ± 0.8	24.8 ± 0.7	25.8 ± 0.8
Total DAD score*	70.4 ± 1.6	71.1 ± 1.5	70.3 ± 1.6

* Values are means ± SEM.

MMSE = Mini-Mental State Examination; ADAS-cog/11 = Alzheimer's Disease Assessment Scale 11-item cognitive subscale; DAD = Disability Assessment for Dementia scale.

the 32 mg/d groups ($p < 0.001$ in both cases). These differences were confirmed using the more conservative ITT analyses ($p < 0.001$ for both doses versus placebo) (see table 2). The differences in change in ADAS-cog/11 scores between galantamine and placebo groups increased over time for both doses ($p < 0.001$).

Improvements in cognitive function over baseline (ADAS-cog/11) appeared within 1 week of reaching a galantamine dose of 24 mg/d and increased after 3 months of treatment ($p < 0.001$ for both galantamine groups versus baseline at both time points). By 6 months, cognitive function improved from baseline by a mean of 1.7 points in the 24 mg/d group ($p < 0.001$) and by 1.6 points in the 32 mg/d group ($p = 0.02$). This improvement from baseline with both galantamine doses was confirmed by the ITT analyses ($p < 0.01$). In contrast, cognitive function declined in the placebo group by a mean of 2.2 points ($p < 0.001$) (see table 2). Patients' *APOE*-ε4 genotype did not appear to influence the effect of galantamine on ADAS-cog/11 score (table 3).

After 12 months of therapy, mean ADAS-cog/11 scores indicated that cognitive function had been maintained relative to baseline in those patients who received galantamine 24 mg/d throughout that period (confirmed by the ITT analysis) (figure 2). Furthermore, this group of patients had a better outcome on ADAS-cog/11 (as measured by change from baseline in ADAS-cog/11 score) than patients who had received placebo for the first 6 months ($p = 0.03$; confirmed by ITT analysis). In those patients who switched from the higher to the lower ga-

Table 2 Primary efficacy outcomes after 6 months

	Placebo group		Galantamine 24 mg/d group		Galantamine 32 mg/d group	
Assessment	ITT (LOCF)	OC	ITT (LOCF)	OC	ITT (LOCF)	OC
ADAS-cog/11 score, mean (SEM) change from baseline	+2.0 (0.45) (n = 207)	+2.2 (0.52) (n = 157)	−1.9 (0.36)‡ (n = 202)	−1.7 (0.45)‡ (n = 131)	−1.4 (0.44)‡ (n = 197)	−1.6 (0.66)‡ (n = 117)
CIBIC-plus score, n (%)	(n = 196)	(n = 159)	(n = 186)	(n = 135)	(n = 171)	(n = 118)
1 = Markedly improved	1 (0.5)	0 (0)	3 (1.6)	1 (0.7)	2 (1.2)	2 (1.7)
2 = Moderately improved	7 (3.6)	7 (4.4)	6 (3.2)	4 (3.0)	4 (2.3)	4 (3.4)
3 = Minimally improved	19 (9.7)	14 (8.8)	28 (15.1)	22 (16.3)	21 (12.3)	17 (14.4)
4 = No change	84 (42.9)	67 (42.1)	99 (53.2) †	68 (50.4) *	91 (53.2) *	57 (48.3) *
5 = Minimally worsened	60 (30.6)	47 (29.6)	36 (19.4)	29 (21.5)	43 (25.1)	30 (25.4)
6 = Moderately worsened	24 (12.2)	23 (14.5)	10 (5.4)	8 (5.9)	9 (5.3)	7 (5.9)
7 = Markedly worsened	1 (0.5)	1 (0.6)	4 (2.2)	3 (2.2)	1 (0.6)	1 (0.8)

* $p < 0.05$.
† $p < 0.01$.
‡ $p < 0.001$ versus placebo.

ITT = intention-to-treat analysis; LOCF = last-observation-carried-forward analysis; OC = observed cases analysis; ADAS-cog/11 = Alzheimer's Disease Assessment Scale 11-item cognitive subscale; CIBIC-plus = Clinician's Interview-Based Impression of Change plus Caregiver Input.

Table 3 Change in ADAS-cog/11 score at 6 months by APOE genotype

	Change in ADAS-cog/11 score, mean (SEM)		
APOE-ε4 allele, n (genotype)	Placebo	Galantamine 24 mg/d	Galantamine 32 mg/d
Two			
(ε4–ε4)	+2.2 (1.3) (n = 27)	−1.8 (1.5) (n = 20)	+0.7 (1.8) (n = 13)
One			
(ε2–ε4 or ε3–ε4)	+2.2 (0.9) (n = 59)	−0.6 (0.6) (n = 55)	−2.4 (1.1) (n = 53)
None (ε2–ε2, ε2–ε3, or ε3–ε3)	+2.1 (0.9) (n = 56)	−2.8 (0.8) (n = 48)	0.0 (0.9) (n = 39)

ADAS-cog/11 = Alzheimer's Disease Assessment Scale 11-item cognitive subscale.

lantamine dose for the extension phase of the study, there was a small deterioration in the ADAS-cog/11 score relative to baseline (mean [SEM], 1.8 [0.86] points; $p = 0.04$) (see figure 2).

Using the CIBIC-plus as a measure of overall clinical response to therapy, 70% of patients on galantamine 24 mg/d and 68% of those on 32 mg/d remained stable or improved over 6 months, compared with only 55% of those in the placebo group (see table 2). Both doses of galantamine produced a better outcome on CIBIC-plus ratings than placebo at 3 and 6 months, which was confirmed by the ITT analyses ($p < 0.05$ for all comparisons). During the 6 months of the extension phase, the proportions of patients who had remained stable or improved, according to CIBIC-plus, were comparable across the three treatment groups (54% to 61%).

Secondary efficacy variables. In the double-blind phase, there were approximately twice as many ADAS-cog/11 responders in the galantamine-treated groups (33.3% and 33.6%) as in placebo-treated group (16.6%, $p < 0.01$ for both comparisons). Galantamine also produced a better outcome on ADAS-cog/13 compared with placebo at 6 months; the treatment effect was 4.5 points for the lower dose and 4.1 points for the higher dose ($p < 0.001$ for both comparisons). Galantamine's advantages over placebo on these secondary outcome measures was confirmed on ITT analyses ($p < 0.01$ for all comparisons on both efficacy measures).

After 6 months of treatment, there were no significant differences between treatment groups in the mean change in total DAD score from baseline. At the end of the extension phase, the change in total DAD score from baseline in patients who had received galantamine 24 mg/d for 12 months was not significant (mean [SEM] decrease of 1.7 [1.78]). DAD cluster scores revealed that both instrumental and basic ADL had been maintained at baseline levels in this group of patients. In contrast, the total DAD score at 12 months in the group of patients who received placebo during the double-blind phase decreased relative to baseline (8.1 [1.94]; $p < 0.001$ versus baseline). The mean [SEM] decrease in total DAD score at 12 months in those patients who had received galantamine 32 mg/d during the double-blind phase was 6.2 (1.71) ($p < 0.001$ versus baseline). All of these findings were confirmed with the ITT analyses.

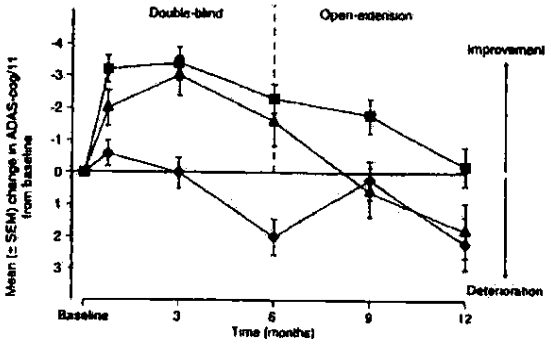

Figure 2. Mean change from baseline in 11-item AD Assessment Scale cognitive subscale (ADAS-cog/11) scores over 12 months (observed cases analysis). ■ = Galantamine 24 mg/galantamine 24 mg; ▲ = galantamine 32 mg/galantamine 24 mg; ◆ = placebo/galantamine 24 mg.

Safety. Adverse events occurring at least 5% more often in either galantamine group than in the placebo group during the double-blind phase are listed in table 4. The majority of adverse events were mild to moderate in severity and predominantly gastrointestinal. Nausea was the most commonly reported event with galantamine. Reports of muscle weakness on galantamine were rare and no more common than that reported with placebo. The proportions of serious adverse events were comparable across treatment groups during the double-blind phase (13% to 16%); these included one death in each group, neither of which was considered related to the study drug by the investigator. During the extension phase, the incidence of treatment-emergent adverse events was low in patients who had received galantamine during the double-blind phase (see table 4). Furthermore, no unexpected, time-dependent, adverse events were reported during the extension phase.

Discontinuations due to adverse events during the double-blind phase were more common in galantamine-treated patients than in those receiving placebo (23% in the 24 mg/d group and 32% in the 32 mg/d group versus 8% with placebo) (see figure 1); 42% (49/116) of galantamine discontinuations due to adverse events occurred during dose escalation, compared with 13% (2/16) in the placebo group. Only 16% of patients withdrew from the extension phase due to adverse events.

There were no clinically significant differences between treatment groups in blood chemistry, hematology, urinalysis, pulse rate, blood pressure, or EKG variables during the double-blind phase. At 6 months, mean body weight had decreased by 2.1 to 2.5 kg in galantamine-treated patients, compared with a slight increase (0.1 kg) in the

placebo group ($p < 0.001$). However, weight loss of 10% or more occurred mostly in patients with moderate to high baseline weights (>50 kg for women and >70 kg for men). Furthermore, the weight loss recovered somewhat during the extension phase with a mean weight loss compared with baseline of only 1.5 kg after 12 months in galantamine-treated patients.

Discussion. The present trial shows that at 6 months, galantamine significantly improved cognitive and global function relative to placebo, and at 12 months cognitive performance and daily functioning were maintained. Over the 12-month study period, galantamine was safe with only a minority of patients discontinuing treatment due to adverse events.

The double-blind phase of the study indicated that galantamine therapy significantly improved cognition compared with placebo, by about 4 points on the standard ADAS-cog/11 subscale. Furthermore, the treatment effect of galantamine relative to placebo increased over the 6-month double-blind period. The data from the ADAS-cog/13 scale and the analysis of responder rates confirmed the benefits of galantamine on cognitive function. At 6 months, the improvement from baseline on ADAS-cog/11 was significant for both doses of galantamine, indicating a sustained improvement in cognitive function during this period. The cognitive decline at 6 months in the placebo group (2.2 points on the ADAS-cog/11 subscale) was comparable with that generally found in placebo groups in other studies.[24-30] There were no clinically significant differences between the 24 mg/d and 32 mg/d doses of galantamine on ADAS-cog/11 or CIBIC-plus, suggesting no additional benefit from the higher dose.

The efficacy of galantamine in this study, as assessed by change in ADAS-cog/11, did not appear to be affected by patients' *APOE* genotype. This result is in contrast to reports of reduced efficacy of tacrine in AD subjects expressing the *APOE*-ε4 allele compared with those not expressing it.[31,32] However, a recent analysis of pooled data from metrifonate studies also suggests that *APOE* genotype does not predict response to treatment.[33] Further investigation of the effects of *APOE* genotype on therapeutic response to other cholinergic treatments would help clarify these divergent findings.

Long-term, placebo-controlled studies are the ideal way to assess the duration of benefit of treatments in AD. However, such studies are difficult to conduct because of ethical reasons and high drop-out rates. An alternative, but less robust, method is to conduct an open-label extension study.[34,35] Data from patients on extended treatment can be compared with either data from untreated patient cohorts or with data extrapolated from the placebo group that participated in the double-blind phase of the study. At 12 months, the group of patients treated with galantamine 24 mg/d had a mean ADAS-cog/11 score that was not significantly different from baseline. The average annual decline in ADAS-cog/11 for untreated AD patients is about 8 points, but the rate of change is less for patients with mild AD.[36] In the present study, linear extrapolation of the change in ADAS-cog/11 in the placebo group at 6 months (2.2 points) suggests a 1 year decline in the placebo group of some 4 to 5 points. These data indicate that galantamine produces clinically significant benefits for at least 12 months.

The group of patients who were switched from galantamine 32 mg/d to 24 mg/d at 6 months demonstrated less benefit in cognitive function than those receiving 24 mg/d for 12 months. Although the reason for this difference in benefit is not clear, it may be that the reduction in dose (from 32 to 24 mg/d) was associated with a rebound effect that led to some loss of efficacy. Patients who received galantamine 24 mg/d for 12 months did significantly better on ADAS-cog/11 than patients who received this dose following 6 months of placebo. This may indicate a beneficial effect of early therapy with galantamine.

Although the double-blind phase did not demonstrate a statistically significant benefit of galantamine on ADL, at the end of the open-label phase there was a strong indication of galantamine's favorable effects on patients' ADL. In the group who received galantamine 24 mg/d for 12 months, daily functioning was preserved, as indicated by a total DAD score that was not significantly different from baseline. This benefit was observed for both basic and instrumental ADL. Functional decline in AD is progressive and, once lost, the ability to perform daily activities is rarely recovered.[37] Moreover, functional disability is an important determinant of caregiver distress and use of healthcare resources.[38] A treatment that preserves patients' functional abilities would be expected to reduce the burden on caregivers and therefore may delay institutionalization.

The proportion of all discontinuations due to adverse events occurring during the dose-escalation phase was greater with galantamine than with placebo. In clinical practice, slow dose escalation may improve compliance with cholinergic agents by minimizing side effects.[39] In a recent 5-month placebo-controlled study, in which the galantamine dose was slowly escalated over an 8-week period to 24 mg/d, only 10% of patients discontinued treatment due to adverse events, which was comparable with the discontinuation rate in the placebo group (7%).[40]

The adverse events reported more commonly in patients receiving galantamine were those expected from cholinergic stimulation. They are generally consistent with those reported in 6 month AD trials of other cholinergic drugs.[24,30] The most common adverse effects of galantamine were gastrointestinal, particularly nausea. However, slowly escalating the galantamine dose, or using a lower dose, has been shown to substantially reduce the frequency of gastrointestinal side effects such as nausea.[40] Reports of muscle weakness on galantamine were rare and no more common than with placebo. Furthermore, the

Table 4 Treatment-emergent adverse events during the double-blind and extension phases

Adverse event*	Double-blind phase			Extension phase
	Galantamine 24 mg/d (n = 212)	Galantamine 32 mg/d, group (n = 211)	Placebo (n = 213)	Patients who received galantamine in double-blind phase (n = 218)
Nausea	79 (37.3)	92 (43.6)	28 (13.1)	23 (10.6)
Vomiting	44 (20.8)	54 (25.6)	16 (7.5)	10 (4.6)
Dizziness	29 (13.7)	39 (18.5)	24 (11.3)	15 (6.9)
Diarrhea	26 (12.3)	41 (19.4)	21 (9.9)	20 (9.2)
Anorexia	29 (13.7)	43 (20.4)	12 (5.6)	12 (5.5)
Weight loss	26 (12.3)	23 (10.9)	10 (4.7)	10 (4.6)
Abdominal pain	14 (6.6)	23 (10.9)	9 (4.2)	13 (6.0)
Tremor	11 (5.2)	7 (3.3)	1 (0.5)	11 (5.0)
Any adverse event	195 (92.0)	195 (92.4)	168 (78.9)	186 (85.3)

Data are expressed as n (%).

* Only adverse events occurring at least 5% more frequently during treatment with either galantamine dose than with placebo during the double-blind phase are shown.

incidence of clinically significant abnormalities on liver function tests for patients treated with galantamine did not differ from patients treated with placebo.

The tolerability of galantamine improved with duration of treatment, and no unexpected adverse events were seen in patients who received 12 months of treatment. These 12-month data, together with its effects on cognitive and daily function, suggest that galantamine produces long-term benefits in the treatment of AD.

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Appendix

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Galantamine in AD

A 6-month randomized, placebo-controlled trial with a 6-month extension

M.A. Raskind, MD; E.R. Peskind, MD; T. Wessel, MD; W. Yuan, PhD; and the Galantamine USA-1 Study Group

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EXHIBIT 27

ORIGINAL CONTRIBUTION

The Cognitive Benefits of Galantamine Are Sustained for at Least 36 Months

A Long-term Extension Trial

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Background: Alzheimer disease (AD) causes progressive cognitive and functional decline over years. Although cholinesterase inhibitors have demonstrated efficacy in studies lasting 3 to 6 months, little is known about long-term therapy.

Objective: To report the long-term cognitive effects of galantamine hydrobromide given continuously for 36 months in AD patients.

Participants: Subjects were 194 US patients with mild to moderate AD who had been randomized to continuous galantamine therapy in either of 2 double-blind placebo-controlled trials. Subjects subsequently received open-label continuous galantamine therapy for up to 36 months.

Main Outcome Measures: Effects on cognition were analyzed as change from study enrollment baseline in scores on the Alzheimer's Disease Assessment Scale–11-item cognitive subscale. Cognitive decline in galantamine-treated subjects was compared with that in a clinically similar historical control sample of AD patients who had received placebo for 12 months and with the mathematically predicted decline of untreated patients over 36 months. The rate of cognitive decline of patients who completed the entire 36-month trial (n=119) was compared with that of patients who withdrew for any reason during the long-term open-label extension (n=75). An inverted responder analysis was also performed in 36-month completers.

Results: Patients treated continuously with galantamine for 36 months increased a mean±SE of 10.2±0.9 points on the Alzheimer's Disease Assessment Scale–11-item cognitive subscale—a substantially smaller cognitive decline (approximately 50%) than that predicted for untreated patients. Patients discontinuing galantamine therapy before 36 months had declined at a similar rate before discontinuation as those completing 36 months of treatment. Almost 80% of patients who received galantamine continuously for up to 36 months seemed to demonstrate cognitive benefits compared with those predicted for untreated patients.

Conclusions: Cognitive decline over 36 months of continuous galantamine treatment was substantially less than the predicted cognitive decline of untreated patients with mild to moderate dementia. Thus, the cognitive benefits of galantamine seemed to be sustained for at least 36 months. These findings suggest that galantamine slows the clinical progression of AD.

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ALZHEIMER DISEASE (AD), the most common cause of late-life dementia, is an inexorably progressive disorder culminating over multiple years in severe disability and death.[1] A deficit in brain cholinergic function contributes to the cognitive impairments of AD.[2] Acetylcholinesterase inhibitors (AChEIs), which enhance cholinergic neurotransmission,[2,3] improve cognition modestly in a subgroup of AD patients and stabilize cognition and function on average for up to 12 months.[4-12] In several AChEI studies,[4-12] patients initially randomized to receive placebo and then given open-label AChEI treatment failed to achieve the same improvement as those initially randomized to receive an AChEI who continued to undergo open-label AChEI treatment. In addition, a retrospective analysis of data from a large trial of tacrine hydrochloride in AD patients suggested that long-term treatment at an adequate dose delayed nursing home placement.[13] These observations indicate that AChEIs may slow the clinical progression of AD. Were this to be demonstrated over multiple years, it would have substantial clinical and public health implications.

Determining the possible long-term benefits of AChEI treatment would require a multiyear placebo-controlled clinical trial. However, ethical considerations

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preclude long-term placebo use. A less direct, yet informative, approach is to determine if AD patients given an AChEI for multiple years manifest more gradual cognitive decline than would be predicted by a mathematical model derived from observations of AD patients followed up longitudinally predating the introduction of AChEIs. The equation of Stern et al[14] predicts subsequent decline among AD patients untreated with AChEIs over multiple years from a given baseline level of cognitive function as quantified by the Alzheimer's Disease Assessment Scale–11-item cognitive subscale (ADAS-cog/11).[15] Because the ADAS-cog/11 is the most common measure of cognition in AD clinical trials, it is possible to compare ADAS-cog/11 score changes observed over time in AD patients treated continuously with AChEIs with changes predicted by the equation of Stern et al.

Galantamine hydrobromide (Reminyl), the most recently approved AChEI, exhibits a dual mechanism of action: competitive acetylcholinesterase inhibition and nicotinic receptor modulation.[3,16,17] In several large phase 3 clinical trials, galantamine demonstrated benefits across all domains of mild to moderate AD (cognition, activities of daily living, behavior, and caregiver burden), with a favorable tolerability and safety profile.[4-7]

Herein, we compared cognitive decline in AD patients treated continuously with galantamine, 24 mg/d, for up to 36 months with the decline predicted by the equation of Stern et al.[14] We also asked if any apparent slowing of decline in patients taking galantamine for 36 months could be attributed to rapid decliners being overrepresented among patients who discontinued treatment at some point during long-term open-label treatment.

METHODS

SUBJECTS

Subjects met the following criteria at enrollment into the original double-blind studies: probable AD based on the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association's criteria,[13] mild to moderate dementia (score of 11-24 on the Mini-Mental State Examination),[18] cognitive decline that was gradual in onset and progressive over at least 6 months, and no clinical evidence of a cause of cognitive impairment other than AD.

STUDY DESIGN

Cognitive function and adverse effects were evaluated in 194 persons with AD who enrolled in a 24-month, long-term, open-label, 24-mg/d galantamine extension study. This study followed participation in either of 2 double-blind placebo-controlled multicenter galantamine trials with continuous open-label galantamine extension for a total original trial galantamine continuous exposure of 12 months. In trial 1,[4] a US placebo-controlled multicenter study, 423 patients were originally randomized to receive galantamine, 24 or 32 mg/d, during the 6-month double-blind phase. Of those patients completing the 6-month double-blind phase, 240 elected to continue open galantamine treatment, 24 mg/d, for an additional 6 months. Of those subsequently completing continuous galantamine treatment after 12 months, 167 patients elected to enroll in the present long-term, open-label, 24-month extension study. Trial 2 was a 6-country international multicenter study.[7] Twenty-seven US participants initially randomized to receive galantamine, 24 or 32 mg/d, during the 3-month double-blind phase, who then were randomized to receive galantamine in a subsequent 6-week washout phase and then completed an additional 7½ months of open-label galantamine therapy, elected to enroll in the present long-term, open-label, extension study. Thus, 194 US patients who received up to 36 months of continuous galantamine therapy, at least 24 mg/d, were included in this analysis. These 194 subjects included 184 white subjects, 6 black subjects, 3 Mexican American subjects, and 1 Asian subject.

OUTCOME MEASURES

Safety evaluations included physical examinations, electrocardiography, vital sign measurements, standard laboratory tests, and adverse event (AE) monitoring.

Effects on cognition were analyzed as ADAS-cog/11 score changes from baseline at double-blind study phase enrollment to that after 36 months of continuous galantamine treatment. Subjects receiving galantamine, 24 mg/d, were compared with untreated patients in 2 ways. The ADAS-cog/11 scores of patients treated with galantamine for the first 12 months were compared with those of a clinically and demographically similar historical control group of mild to moderate AD patients who had received placebo for 12 months in an earlier multicenter trial. All placebo-treated subjects of the earlier trial whose baseline ADAS-cog/11 score was within 1 SD of the baseline ADAS-cog/11 score of the present subjects receiving continuous 36-month galantamine therapy comprised the historical control group. There was no significant ($P=.46$) difference in baseline ADAS-cog/11 scores between these groups. The 194 galantamine-only–treated subjects in the present study did not differ significantly from the 186 subjects in the historical placebo group by sex (females, 56.7% vs 56.6%; $P=.88$) or age (mean±SE, 76.1±0.52 vs 74.1±0.52 years; $P=.19$).

The slope of ADAS-cog/11 scores over time of subjects receiving galantamine, 24 mg/d, continuously for up to 36 months was compared with that of mathematically predicted ADAS-cog/11 scores of untreated subjects.[14] Stern and colleagues[14] determined the quadratic relationship between ADAS-cog/11 scores and the annual rate of cognitive decline in AD patients followed up longitudinally before the introduction of cholinesterase inhibitor treatment. After 3 years, the ADAS-cog/11 scores of untreated patients at a comparable initial level of dementia as that of the actual galantamine-treated subjects at study enrollment were predicted to increase by 20.5 to 22.0 points.[14] Also, the validity of the predicted slope of ADAS-cog/11 scores over time of untreated patients was estimated by comparing it with the slopes of the 6-month double-blind placebo group in trial 1 and of the 12-month historical placebo group.

An inverted responder analysis was performed for the 119 patients who completed 36 months of continuous galantamine treatment. The percentage of responders was categorized as follows: 0-point or fewer increase, 4-point or fewer increase, 7-point or fewer increase, 10-point or fewer increase, and 20-point or fewer increase on the ADAS-cog/11. This analysis was used to evaluate the number of patients who, after receiving galantamine continuously for 36 months, had better cognitive function than that predicted for untreated patients.

STATISTICAL ANALYSIS

The efficacy analysis was a traditional observed-cases analysis. The baseline visit of the 3- or 6-month double-blind study was used as the baseline examination in this study. Assessment times (time from baseline of the original double-blind studies) were months 12, 18, 24, 30, and 36. The main analysis was based on change from baseline to month 36. Within-group comparisons for the change from baseline or the initial visit were performed using the paired t test.

Table 1. Demographic and Baseline Characteristics of the Groups*

Characteristic	All Patients (N = 327)	Galantamine-Treated Patients (n = 194)	Historical Placebo Group (N = 166)†
Sex			
Male	144 (44.0)	84 (43.3)	81 (43.5)
Female	183 (56.0)	110 (56.7)	105 (56.5)
Race			
Black	8 (2.4)	6 (3.1)	NA
White	306 (94.2)	184 (94.8)	NA
Mexican American	7 (2.1)	3 (1.5)	NA
Asian	3 (0.9)	1 (0.5)	NA
Other	1 (0.3)	0	NA
Age, y‡	76.2 ± 0.40	76.1 ± 0.52	74.1 ± 0.62
MMSE score‡	19.8 ± 0.20	19.7 ± 0.27	18.8 ± 0.19

Abbreviations: MMSE, Mini-Mental State Examination; NA, not applicable.
*Data are given as number (percentage) of each group unless otherwise indicated. Percentages may not total 100 because of rounding. $P = .39$ between the 3 groups. Galantamine was given as galantamine hydrobromide.
†Described by Torris.[19]
‡Data are given as mean ± SE.

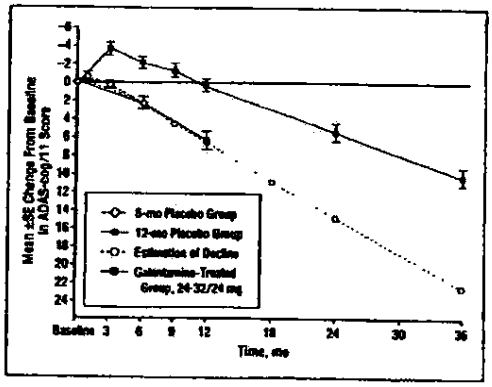

Figure 1. Change from baseline in Alzheimer's Disease Assessment Scale–11-item cognitive subscale (ADAS-cog/11) scores of patients treated with galantamine hydrobromide for 36 months. The 6-month placebo group was described by Raskind et al[4]; the 12-month placebo group, by Torris[19]; and the estimation of decline, by the equation of Stern et al.[14] The number of galantamine-treated patients was as follows: baseline, n = 192; month 3, n = 189; month 6, n = 184; month 9, n = 159 (not all patients had an efficacy value this month); month 12, n = 186; month 24, n = 140; and month 36, n = 90 (while 119 patients continued to receive therapy, efficacy values for only 90 were available).

Also, it was recognized that the less-than-predicted cognitive decline in subjects treated with galantamine for 36 months might be a function of having lost the more rapidly declining subjects who discontinued treatment. To assess the effect of discontinuation over 36 months on overall cognitive efficacy results, a random coefficient model was used to analyze dropouts. Changes in ADAS-cog/11 scores for each patient were calculated and applied to a *t* test comparing the slope and intercept of a line depicting the change for the entire group and for each patient as a random subject. This analysis was performed using a random coefficient model to compare the slopes of the completer and discontinued populations, using the last 2 observed values for all patients.

Table 2. Adverse Events Occurring in 10% or More of Patients Receiving Open-Label Galantamine*

Adverse Event	All Patients (N = 327)	Galantamine-Treated Patients (n = 194)
Agitation	87 (26.6)	51 (26.3)
Urinary incontinence	63 (19.3)	46 (23.7)
Fall	55 (16.8)	35 (18.0)
Depression	52 (15.9)	32 (16.5)
Insomnia	49 (15.0)	27 (13.9)
Anorexia	48 (14.7)	27 (13.9)
Urinary tract infection	47 (14.4)	22 (11.3)
Injury	45 (13.8)	26 (13.4)
Weight decrease	41 (12.5)	28 (14.4)
Dizziness	40 (12.2)	28 (14.4)
Confusion	36 (11.0)	25 (12.9)

*Data are given as number (percentage) of each group. Galantamine was given as galantamine hydrobromide.

RESULTS

The demographic and baseline characteristics of all groups are given in **Table 1**. One hundred ninety-four US patients who had received continuous galantamine therapy from the inception of trials 1 and 2 were enrolled into the multiyear long-term extension study. Of these patients, 119 (61.3%) completed the 36-month study. The most common reasons for discontinuation were AEs and withdrawal of consent. Adverse events occurring in 10% or more of patients treated with galantamine for up to 36 months are listed in **Table 2**.

SAFETY

Galantamine, 24 mg/d, was well tolerated when administered for 36 months. Most AEs observed were transient, of mild to moderate intensity, and qualitatively similar to those of previous trials.[4-7] However, the nausea and vomiting observed frequently in short-term trials were uncommon in this study. The AEs seen most frequently were psychiatric disorders (65.7%) characteristic of an elderly AD population followed up for 3 years (agitation, insomnia, and depression).[20-22] Few AEs were rated severe. The most common severe AEs were pneumonia (3.7%), falls (3.4%), and injury (3.1%). There were no clinically relevant changes in laboratory values, vital signs, or electrocardiographic readings.

COGNITION

The change from baseline in ADAS-cog/11 scores for patients receiving galantamine for 36 months is illustrated in **Figure 1**. The ADAS-cog/11 scores at 12 months did not differ from baseline, whereas the ADAS-cog/11 scores of patients in the historical placebo group increased by a mean ± SE of 6.26 ± 0.54 points. Patients continuously treated with galantamine, 24 mg/d, did not experience a cognitive decline similar to that in the historical placebo group at 12 months until month 24. Furthermore, the ADAS-cog/11 scores of patients continuously treated with galantamine over 36 months increased by only a mean ± SE

of 10.2±0.9 points vs the 20.5- to 22.0-point increase predicted by the equation of Stern et al.[14] Patients continuously treated with galantamine maintained ADAS-cog/11 scores at or above baseline for the first 12 months, and gained approximately 18 months in preservation of cognition relative to the equation of Stern et al.

The slope of cognitive decline observed during 36 months of continuous galantamine treatment was significantly less steep than that predicted by the equation of Stern et al[14] ($P=.03$). In contrast, the decline over 6 months of the trial 1 placebo-treated subjects and the decline over 12 months of the historical placebo-treated subjects were almost identical to that predicted by the equation of Stern et al. Assuming that these slope similarities would persist if the placebo groups had been followed up for 36 months, these findings support the validity of the decline predicted by the equation of Stern et al as a comparison for that observed over 36 months of continuous galantamine therapy.

Results of the inverted responder analysis, presented in **Figure 2**, reveal that 21 (17.6%) of the 119 patients who received continuous therapy with galantamine, 24 mg/d, for 36 months maintained cognitive function at or above baseline (ADAS-cog/11 score unchanged or decreased). More than half of the patients had an increase of 10 or fewer points on the ADAS-cog/11 after 36 months, substantially less than the expected decline in cognitive function (≤20-point increase on the ADAS-cog/11) for untreated AD as predicted by the equation of Stern et al.[14]

The positive effects of long-term galantamine treatment could not be attributed to differential rates of decline between those who completed and those who withdrew from the study. The comparison of slopes for the completed and discontinued populations demonstrated that the 181 patients who completed the study were not statistically different ($P>.40$) from the overall study population, including patients who discontinued therapy for any reason ($n=144$) (**Figure 3**).

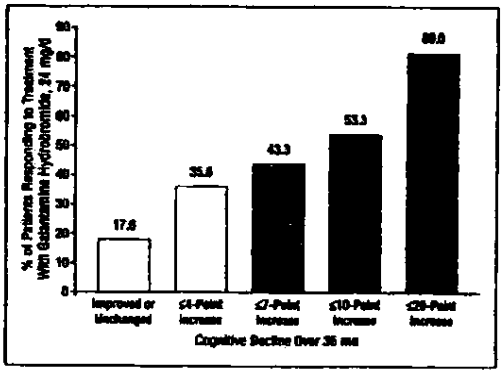

Figure 2. Percentage of patients responding to 36 months of galantamine hydrobromide, 24 mg/d, treatment ($n=119$). Cognitive decline after 36 months is estimated to increase by 20.5 to 22.0 points in untreated patients, as measured by the Alzheimer's Disease Assessment Scale–11-item cognitive subscale.[14]

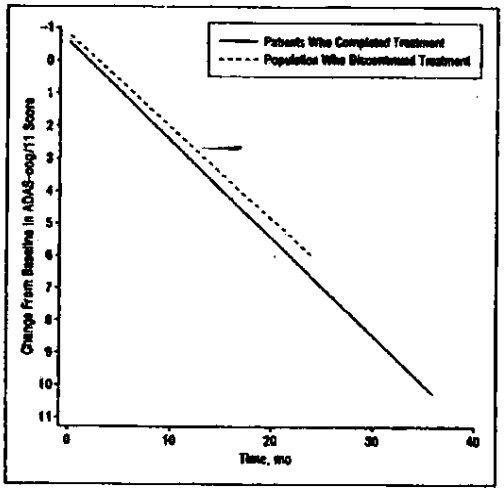

Figure 3. Change from baseline in Alzheimer's Disease Assessment Scale–11-item cognitive subscale (ADAS-cog/11) scores in the overall study population, including those with discontinued treatment, vs patients who continued to receive galantamine hydrobromide treatment—calculated using the random coefficient model for the last 2 measurements. $P>.40$ between slopes.

COMMENT

These data support the hypothesis that continuous galantamine treatment slows the rate of cognitive decline in AD patients for up to 3 years. On average, patients treated with galantamine, 24 mg/d, maintained cognitive function at pretreatment baseline levels for the first 12 months of therapy. At end point, cognitive decline in galantamine-treated patients was delayed by approximately 18 months vs the predicted decline in untreated AD patients. Almost one fifth of patients had cognitive function at or above prerandomization levels by the end of 36 months of galantamine treatment, and greater than 50% demonstrated improvement in cognition vs that projected for untreated patients. The similarity between the slopes of ADAS-cog/11 score decline in the historical placebo group and as predicted by the equation of Stern et al[14] supports the validity of this mathematical modeling approach for estimating cognitive decline in AD patients. Because untreated AD patients show progressive cognitive decline, maintaining cognitive function at baseline levels or delaying cognitive decline should be viewed as treatment benefits. Together with findings from other studies,[4,12] these results strengthen the argument for early diagnosis and treatment and support the hypothesis that AChEI treatment slows AD progression.

Enhancement of cholinergic neurotransmission might slow progression in AD patients by several possible mechanisms. Muscarinic acetylcholine receptor stimulation favors nonamyloidogenic processing of amyloid precursor protein in cell lines and primary neuronal cultures.[23] Muscarinic acetylcholine receptor stimulation also reduces phosphorylation of the cytoskeletal protein tau in pheochromocytoma 12 cells transfected with M1 muscarinic acetylcholine receptors.[24] Allosteric modulation of nicotinic acetylcholine receptors (nAChRs) by galantamine also could slow AD progression. The density of nAChRs decreases in AD patients.[25,26] Loss of nAChRs is strongly correlated with AD severity.[16,26-29] Stimulation of nAChRs inhibits the neurotoxic effects of β-amyloid in cultured neurons; this effect

is mediated by the $\alpha_4\beta_2$ nAChR widely present in the cerebral cortex,[30] and improves learning, memory, and attention.[31-34] Furthermore, autoradiographic, histochemical, and brain imaging studies in AD patients indicate that nAChR loss is more severe than loss of muscarinic acetylcholine receptors or choline acetyltransferase. Galantamine's ability to modulate nAChRs,[17] and inhibit acetylcholinesterase, may contribute to its broad spectrum of therapeutic benefits and sustained effectiveness in AD patients.[35]

Galantamine seemed effective and was safe and well tolerated in AD patients with mild to moderate dementia for 36 months. Although these results suggest long-term positive effects on clinical decline in AD patients treated with galantamine, conclusions are limited by the lack of biological indicators of disease progression and the absence of a true long-term placebo control group. Even more convincing demonstrations of disease-modifying effects of AChEIs hopefully will emerge from ongoing placebo-controlled trials in persons with mild cognitive impairment.

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EXHIBIT 28

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CHAPTER 26

Clinical strategies in the treatment of Alzheimer's disease

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Introduction

Every culture has tried to obtain life extension. A 2400-years-old example is the Babylonian-Assyrian epos of Gilgamesh, who tried in vain to escape the aging process by bathing in the fountain of youth and by eating the herb of life. The latter endeavor makes clear that 'research' in the pharmacological prevention of the symptoms of aging has a long history. From the pharmacological literature of the last century it is evident that hypotheses concerning the causal factors of aging and Alzheimer's disease as well as the therapies for treating them were often changing parallel to neurobiological interests and fashions. Clinical strategies were not specific for Alzheimer's disease, but passively followed new developments in medicine and research by trying out nearly every new compound or idea relevant to this condition. This may also explain why therapies that were considered to be 'rational' in the light of a new development, appeared nonsensical as soon as new insights developed.

The approach of trying out everything that is new in neurobiology on Alzheimer patients does not preclude of course the possibility either that an effective substance will be found, or that its proposed mechanism of action will indeed turn out to be correct. However, the chance of success of this strategy is very small. Repeatedly, ideas about the etiology of Alzheimer's disease have been adapted immediately to new disciplines or insights that developed in neurosciences. Thus changes in hormone levels, blood supply, metabolism, and transmitters have been pinpointed as possible causes of brain aging and Alzheimer's disease. Subsequently, a 'new and promising' therapy was claimed to have a 'rational' basis and was tried out on Alzheimer patients. This history might make us less optimistic about all the ongoing clinical trials, and even more convinced about the necessity of fundamental research in Alzheimer's disease before a therapy with a reasonable chance of success will ever succeed in being developed.

Experimental endocrinology started in 1848 when Berthold, professor of medicine at the University of Göttingen, showed that the atrophy of the comb and the changes in behavior following castration of cockerels, could be prevented by transplantation of testis (cf. Tausk, 1976). Endocrine experiments in animals were followed by series of observations on the possible effects of gonadal hormones on the process of aging and dementia in man. Brown-Séquard (1889), at the age of 72, injected himself with extracts prepared from crushed testicles of guinea-pigs or dogs. We may wonder now how little of the active steroids these aqueous extracts must have contained. Yet he claimed that both his physical and intellectual powers increased. Lorand (1913) reviewed the 'marvelous effects' of ovarian extracts, thyroid extracts and extracts of testicles on the prevention and treatment of the symptoms of old age. Lorand too ('for experimental purposes', as he explained, apparently being in need of an apology) subsequently tried out testicular

extracts from the pig on himself and confirmed the increase in 'muscular and mental' powers. In the line of thought of that period, transplantation of animal testicular tissue to the testis of aged men was quite logical. This treatment, with monkeys as donors was indeed reported to be very successful in old animals including man (Voronoff, 1925).

In the same period an indirect way of increasing gonadal hormone levels was proposed by Steinach. He claimed to have experimental evidence for 'hormone accumulation' by ligation of the vas deferens, an operation that would result in 'reactivation'. He subsequently asked the Viennese surgeon and urologist Dr. Robert Lichtenstern "to performe vasoligature on suitable patients for reactivation purposes". Lichtenstern carried out the first 'Steinach' operation on November 1, 1918 on the vas deferens of an 'exhausted and prematurely old man'. This operation was followed by 'many thousands — perhaps even tens of thousands — of successful repetitions'. Steinach called the operation "a means of enriching our stock of remedies against pre-senility, inasmuch it removes disturbances of the central nervous system" (Steinach and Loebel, 1940). Reading through their case histories labelled as 'premature senility', 'moods of depression' are mentioned remarkably often. In those days, the differential diagnosis between depression and dementia will already have been a difficult one, and a beneficial effect of this operation upon depression might be the explanation of the astonishing high success rate of some 80%. It is a pity that Steinach's misconception may have contributed to preventing serious study on the effects of testosterone treatment on aging subjects, since, at present, we know that testosterone levels are indeed decreasing during senescence (Deslypere and Vermeulen, 1984; Warner et al., 1985). Although we do not have any data on testosterone levels in dementia, changes would probably have strong effects on several transmitter systems in the brain. In the old rat, decreased testosterone levels seem to be the most probable explanation for the diminishment of vasopressin-containing fibers originating, e.g., from the bed nucleus of the stria terminalis (Fliers et al., 1985a), although experimental confirmation has still to be performed. Whether or not similar testosterone-dependent fiber systems do also exist in the human brain is not known.

In the subsequent period in our story, the condition of the blood vessels was put central, as appears from the slogan 'a man is as old as his blood vessels' (e.g. Foley, 1956). The idea that dementia was caused by arteriosclerosis of the cerebral vessels led to the development of 'vasodilatators' (see below). Hyperbaric and normobaric oxygen therapy also fit into the vascular hypothesis of aging (McFarland, 1963) and dementia. The initially reported improvements obtained with the use of these therapies were not confirmed in later studies (Wittenborn, 1981; White et al., 1975).

In the meantime, psychotropic drugs had been developed that appeared to be effective, e.g., in treating schizophrenia and depression. They were subsequently applied, without any beneficial effects, in dementia. Such medicines were followed by the 'geronto-psychiatric drugs' that would be 'specifically' beneficial for the elderly patient with mental impairments, again without great success, however.

The recent boom in our knowledge concerning neurotransmitters has resulted in a new direction in gerontological treatments viz. transmitter substitution therapies. In addition, possibly under the influence of social sciences on medicine, increasing attention is currently being paid to non-pharmacological therapies, such as the effects of food or environment (Lieberman and Abou-Nader, 1986; Roth et al., 1986). However, in spite of all these efforts, we still have no effective therapy for

Fig. 1. (a) Old ram (No. 14) in 1918, before transplantation. (b) The same old ram (No. 14) in 1923, five and a half years after transplantation. (c) Operation on a human: one of the grafts, obtained from a monkey testicle, is fixed by four sutures of catgut in the left sinus, the glandular side facing the tunica vaginalis. (d) M.T. 74 years old in 1923, before transplantation. (e) M.T. 76 years old in 1925, two and a half years after transplantation. (From Voronoff, 1925)

Fig. 1.

Alzheimer's disease, which is perhaps not surprising, since its etiology has not yet been elucidated. There may in fact not even exist any such thing, in view of the possibility that Alzheimer's disease may represent an accelerated form of the normal aging process. Such ignorance merely stresses the importance of more fundamental research into the process underlying normal aging of the brain, and Alzheimer's disease in particular. The modest beneficial effects that have been reported for various pharmacological and non-pharmacological therapies and that are reviewed in the present paper might provide some clues for effective further research.

Cerebral vasodilatators

The use of cerebral vasodilatators was based upon the assumption that dementia was largely caused by cerebral arteriosclerosis. The justification for the use of such drugs in Alzheimer's disease is at present weak. Moreover, even if AD were to have a vascular cause and vasodilatators were effective, one could wonder how arteriosclerotic arteries would be able to dilate (Branconnier and Cole, 1977; Yesavage et al., 1979).

Carbon dioxide and *carbonic anhydrase inhibitors* (e.g., acetazolamide) have been used in dementia without effective therapeutic consequences (Ban, 1978; Cole and Liptzin, 1984).

Papaverine, an ancient drug found in opium and having morphine-like analgesic activity, and the related drug *cyclandelate* have been among the most widely prescribed categories of agents in the treatment of 'arteriosclerotic' dementias. It is questionable, whether the reported therapeutic effects, e.g. in elderly volunteers (Branconnier and Cole, 1977; Wittenborn, 1981), were indeed due to improved cerebral blood flow or alternatively; the improved cerebral blood flow is more likely to have been secondary to increased brain metabolism. There is no conclusive evidence that *nicotinic acid*, *tocopherol* (vitamin E) or any of the numerous other 'vasodilating agents' improve either cerebral blood flow or the condition of the Alzheimer patient (Ban, 1978). *Hydergine*, that was developed as a vasodilatator will be discussed in the section on CNS stimulants.

Dicumarol, *warfarin* and other *anticoagulants* have been employed because of the theory that cerebral emboli could contribute to the development of dementia. The studies reported with this therapy were usually uncontrolled and very limited, so that useful conclusions are hard to draw. The risk of bleeding with this therapy is substantial (Ban, 1978; Wittenborn, 1981).

Classical psychotherapeutic drugs

The success of psychotherapeutic drugs in psychiatry has led to their prescription in aging and dementia, where they have to be considered as merely symptomatic agents (Hollister, 1985). Yet, some 36% of the US subjects over 60 years of age have used such drugs (Epstein, 1978). During the last few years, the number of studies on the effect of major tranquilizers on senile patients seems to have diminished (Wittenborn, 1981), presumably because it has become apparent that they have no unequivocally favorable effects on the condition, although the symptomatic improvements may be highly valued by those charged with the care of such patients (cf. Hollister, 1985). On the other hand, this population is certainly at risk for iatrogenic illness secondary to the action of psychotherapeutic drugs.

Antidepressants, neuroleptics and anxiolytic agents

The fact that depression may easily be confused with dementia does of course not mean that *antidepressants* have a favorable action on the clinical condition in Alzheimer's disease. Negative effects have been obtained with such compounds as *neuroleptics*: compounds that even frequently have been the cause of pseudodementia in aged patients (De Beer and Simons, 1977).

Benzodiazepines, *propanediols* and *barbiturates* are frequently used in the elderly and are clearly effective in treating such symptoms as anxiety,

tension, restlessness and agitation. However, there is no indication that tranquilizers improve impaired functioning in old people (Wittenborn, 1981). Moreover, benzodiazepines are among the most frequently misused drugs (Epstein, 1978) and may induce similar amnestic performance deficits as found in Alzheimer's disease (Wittenborn, 1981; Bartus et al., 1982). Long-term use of benzodiazepines might possibly even cause some degree of brain atrophy, since it has been reported to be accompanied with an increased ventricle/brain ratio. However, a causal relationship between drug use and brain atrophy in human has not yet been proven (Lader and Petursson, 1983).

Alcohol is most probably the most widely used anxiolytic compound. Wine or beer in modest amounts improved the condition of old subjects, also in chronic brain syndromes, whereas no difference was found when drinks were given either in a pub or a ward setting (Chien, 1971; Chien et al., 1973). Yet, in the long run, alcohol has serious negative effects, e.g. on gnostic functions (Freund, 1982; Freund and Butters, 1982) and may cause brain atrophy (Lader and Petursson, 1983). Therefore, it cannot be recommended as a safe alternative therapy.

Central nervous system stimulants

Because of the changing ideas about the etiology of dementia through the years and the technical improvements that have enabled the measurement of brain metabolism, pharmacotherapeutical interest shifted from the improvement of cerebral circulation to the improvement of brain metabolism in the elderly. This development has led to many claims regarding new gerontopsychiatric drugs. However, only few weakly effective compounds were in fact produced, presumably because the diminished metabolism in the Alzheimer brain is an effect rather than a cause of the condition (Frackowiak, 1986).

Piracetam (2-oxy-1-pyrrolidine acetamide), originally developed as a compound against motion illness, was later claimed to protect the brain against oxygen shortage and to improve learning. It is a GABA-derivate without GABA effects (Cole and Liptzin, 1984). It was considered to be the first compound of a new class of 'nootropic' drugs (Ban, 1978), i.e., able to enhance memory and learning, and thus of possible importance for the treatment of Alzheimer. This idea was confirmed by a number of methodologically imperfect clinical trials. Careful studies, using a standardized factor-analyzed rating scale for elderly patients (BOP), psychometric tests and a double-blind crossover design did not show any significant effect as compared to placebo, upon psychometric performance of Alzheimer patients (Diesfeldt et al., 1978; Wittenborn, 1981). The drug is currently advertized as Nootropil® for transient ischemic attacks and would — according to the advertisement — improve the disturbed microcirculation and the oxygen and glucose utilization. It has been claimed to be effective in Alzheimer's disease in combination with choline (see below).

Magnesium pemoline (Cylert®) was originally introduced as a compound that would increase the synthesis of ribonucleic acid and, consequently, the consolidation of memory. This finding was, however, shown to be in error (Eisdorfer et al., 1968). The favorable results on memory could not be confirmed in subsequent clinical investigations using tests involving learning, memory, and performance. The compound is now marked for use in children suffering from minimal brain dysfunction and/or hyperkinetic behavior (Branconnier and Cole, 1977; Ban, 1978; Wittenborn, 1981).

Yeast RNA taken orally was supposed to affect memory but — perhaps not too surprisingly — had no better effect than did placebo in old impaired or demented patients (Wittenborn, 1981).

Anabolic agents, such as fluoxymesterone, isoprinosine and related hormone preparations, have been administered to gerontopsychiatric patients in the hope of correcting the disturbance of protein synthesis encountered in aging, however, without any clear-cut effect on memory function (Ban, 1978).

Pentylenetetrazole and *methylphenidase* do not

seem to have an apparent value in improving mental functions (Ban, 1978; Wittenborn, 1981; Cole and Liptzin, 1984). The results with *pipradol* seem to be favorable only in the first weeks of the treatment, but not at subsequent assessment periods (Wittenborn, 1981).

Procaine was introduced in 1956 as 'a new method for prophylaxis and treatment of aging' by Dr. A. Aslan from Roumania supposedly having 'eutropic and rejuvenating effects'. In 1958 she started treatment with *Gerovital H3* (2% procaine-HCl combined with a preservative plus an antioxidant) a preparation which she called *Aslavital*® and for which novel pharmacological properties were claimed. Expensive trips to Roumania are still advertized emphasizing the "remarkable value of Dr. Aslan's cure" that "is efficient in the prophylaxis and cure of the phenomena that appear in the affections of the central nervous system...". In addition, "...it has a favorable effect in...memory, attention and concentration capacity troubles...in the decline of intellectual and physical ability". However, most studies provide little support for the claim that this drug improves the mental status of geriatric patients (Wittenborn, 1981; Millard, 1984). An exception is a study by Hall et al. (1983), that reported an effect on consolidation of new learning and muscle strength, but also documented several adverse reactions. Gerovital H3 probably acts, however, as a mild antidepressant drug, because it is a weak, reversible and competitive inhibitor of MAO (Zung et al., 1974; Branconnier and Cole, 1977).

Hydergine® is composed of the methylates of four dihydrogenated ergot derivates. In the period that the decline of cognitive function in aging and Alzheimer's disease was thought to be due to vascular changes, hydergine was developed and advertized as a vasodilator. Evidence for such an effect is totally lacking. Yet, it is still used in various countries even for the treatment of hypertension (Hollister and Yesavage, 1984) in the belief that it possesses a vasodilatory action. Hydergine was subsequently classified as 'a metabolic enhancer', since in some pharmacological tests it induced a changing in cyclic-AMP levels. How such effects relate to Alzheimer's disease is not at all clear (Hollister and Yesavage, 1984). Recently, the action of hydergine has been explained by its binding to dopamine, serotonin and noradrenaline receptors, or was simply called 'a rational approach' (cf. Ermini and Markstein, 1984). This clearly illustrates how time after time the commercial machinery gets its hand on whatever neurobiological approach is in fashion at the moment. It is no less than amazing that the interesting observation of Nandy and Schneider (1978) that hydergine causes a decrease in lipofuscin content as well as an increase in neurite formation in mouse neuroblastoma cells kept in culture, has not been used in advertisements, since on theoretical grounds such a general effect might prove to be beneficial in the treatment of Alzheimer's disease (cf. Coleman and Flood, 1986). This observation may point to a non-specific metabolic activation of neurons and might as such be an alternative explanation for its effects (see below). Regardless of the validity of the various explanatory proposals, double-blind studies of hydergine versus papaverine-hydrochloride or other controls indeed favored the former, also in cognitive tests, although the improvements were relatively modest. Alzheimer patients were those who benefited the most, provided their condition was not too far advanced, while patients with multi-infarct dementia improved less (Loew and Weil, 1982). The generally reported improved mood and feeling of well-being resulting from hydergine are more pronounced than are the reported cognitive improvements. One may wonder, therefore, whether its effects might not best be explained by an antidepressive action (Fliers, 1982), although some correlations plead against this possibility. As an alternative mechanism of action the induction of decreasing prolactin levels by hydergine are mentioned (Loew and Weil, 1982). Although statistically present, the reported improvements with hydergine are clinically marginal and lacunar, while great improvement in memory has never been observed (e.g., Pomara et al., 1983; Cole and Liptzin, 1984). This consider

ation puts question marks to the clinical usefulness of this drug in the treatment of Alzheimer's disease (Meier-Ruge, 1983; Hollister and Yesavage, 1984). The combination of hydergine with lecithin was not effective in Alzheimer's disease (see below).

Nafronyl, a new compound that would increase metabolic activity and was claimed to have beneficial clinical effects on dementia patients, is currently under further investigation (Yesavage et al., 1982).

Neurotransmitter substitution therapies

At present, the study of specific neurotransmitter systems is a hot topic in neurobiology. No wonder, thus, that various neurotransmitter substitution therapies are currently being proposed for Alzheimer's disease. Neurotransmitters may be subdivided into acetylcholine, monoamines, amino acids and neuropeptides. All four classes of transmitter systems undergo changes during aging and in Alzheimer's disease; findings that have stimulated clinical trials aimed at their substitution in Alzheimer's disease.

Cholinergic system

The 'cholinergic hypothesis' concerning the etiology for the decrease of cognition in the elderly and in Alzheimer's disease has gained considerable attention during the last years (for review see Bartus et al., 1982). Indeed, choline acetyltransferase (CAT) activity and acetylcholine production is markedly reduced in Alzheimer's disease. Moreover, a severe loss of neurons was found in this condition in the nucleus basalis of Meynert, the main source of neocortical cholinergic innervation. Yet it is questionable whether this is indeed an adequate explanation for the etiology of Alzheimer's disease, since disruption of this cholinergic system in the rat causes only a temporary cognitive impairment (Bartus, 1986), whereas lesions in the rat cerebral cortex induce degenerative changes in Meynert's nucleus (Sofroniew et al., 1986). In addition, many other transmitter systems are affected in Alzheimer's disease (Gottfries, 1986; Swaab et al., 1985, 1986; Fliers and Swaab, 1986; Francis et al., 1985).

Clinical studies, aimed at substituting the cholinergic deficit in Alzheimer, have attempted (1) to enhance the synthesis and release of acetylcholine by providing abundant amounts of precursor substances, such as *lecithin* and *choline*, and (2) to enhance cholinergic activity by giving drugs that interfere at the synaptic or postsynaptic site or (3) by inhibiting acetylcholine breakdown of the endogenous transmitter using *physostigmine*. The reported effects of precursors on cognition are generally far from impressive or sometimes even completely negative. There are, however, a few more optimistic reports (Bartus et al., 1982; Drachman et al., 1982; Hollister, 1985). In addition, the dose range seems to be very narrow and to vary considerably among individual subjects (Bartus et al., 1982). The muscarinic agonist *arecoline* may enhance performance on a memory task in Alzheimer's disease, although not to the extent of achieving any significance (Palacios and Spiegel, 1986). Combinations with central nervous system stimulants have also been tried: *choline-piracetam* and *lecithin-piracetam* combinations were reported to be effective in an open trial and in preliminary results of a double-blind cross-over study, respectively (Bartus et al., 1981; Samorajski et al., 1985), but a *hydergine-lecithin* combination was not (Pomora et al., 1983).

In conclusion, although "some clinical improvement can occasionally be seen" (Barbeau, 1978), a satisfactory treatment of the cognitive impairment of Alzheimer's disease by means of pharmacological substitution for deficits in the cholinergic system seems, at present, not to be feasible.

Amines

Recent evidence for considerable cell loss in the locus ceruleus with normal aging and in Alzheimer's disease (Bondareff, 1982), and data on monoamines in brain and CSF, point to catecholamine impairment in the cognitive disturbances

(Gottfries, 1986). Noradrenaline concentrations in the temporal cortex of Alzheimer patients are reduced, as is the serotonin concentration in the frontal cortex, temporal cortex and limbic areas (Francis et al., 1985).

Bromocryptine, a dopamine agonist, has no demonstrable effect on intellectual functioning in Alzheimer patients (Smith et al., 1979). L-*Dopa*, *tyrosine*, *5-hydroxy-tryptophan* and L-*tryptophan* have all been tried in small samples of patients, occasionally leading to a mild improvement (Cole and Liptzin, 1984). In general, however, compounds influencing the aminergic system have not shown any beneficial effect on cognition or mood superior to that of antidepressants (Reisberg et al., 1983a). For a discussion of the proposal that hydergine is effective by virtue of its action on aminergic systems the reader is referred to Ermini and Markstein (1984) and to p. 418 of the present paper.

Amino acids

Drugs influencing this class of transmitters, e.g. the *benzodiazepines*, do not seem to have a favorable action on cognitive functions (see above). Recently, the Japan Economic Journal reported that Chugai Pharmaceutical Co. researchers are testing dibenzoxazepine. It would improve learning in aged rats. They predict that this substance will be effective against Alzheimer's disease. We shall wait and see.

Neuropeptides

Various neuropeptides were first known as hypothalamic hormones (vasopressin, oxytocin, LHRH, TRH, CRF) or pituitary hormones (peptides of the opiomelanocortin family). Their endocrine history and the data on their central effects have led to the concept that the brain, like the peripheral endocrine glands, is an endocrine target organ. Many of the peptides in the brain show changes with aging (De Wied and Van Ree, 1982; Swaab, 1982; Facchinetti et al., 1984; Fliers and Swaab, 1986). Moreover, since functions that are influenced by neuropeptides such as motivational, attentional and memory processes tend to decline during aging (Jolles, 1986a), it was postulated that a decreased bioavailability of neuropeptides in the brain of elderly people is associated with specific disturbances in their mental performance (De Wied and Van Ree, 1982). However, neuropeptides appeared not to act centrally as hormones but rather to be transported throughout the brain by extensive fiber systems which terminate on other neurons by means of synapses that cannot be distinguished from those containing the classical neurotransmitters (Buijs and Swaab, 1979; Swaab, 1982). In spite of the relatively short period of research devoted to them, many neuropeptides already fulfill quite some of the accepted transmitter criteria (Buijs, 1982).

Because of the presumed effects of *vasopressin* on memory consolidation in animal studies, the memory disorders commonly observed in the elderly, and a presumed deficiency of neurohypophyseal hormone release into the periphery during aging, Legros (1975; Legros et al., 1978) studied the influence of vasopressin in men aged 50–65 years and reported a positive effect in memory tests. In later studies, however, less favorable results were obtained (cf. Jolles, 1986b).

From our measurements at the hypothalamic sites of production of vasopressin, the supraoptic (SON) and paraventricular nucleus (PVN), and from the recently reported increased vasopressin blood levels in the aged (cf. Fliers et al., 1985b; Hoogendijk et al., 1985), it has become clear that the vasopressin 'substitution' therapy in elderly, and maybe even in Alzheimer patients, has probably been given to subjects in whom neurohypophyseal function was not deficient at all. On the contrary, vasopressin cells were found to be activated in these conditions, probably by way of compensation for decreased renal sensitivity to vasopressin (E. Goudsmit et al., personal communication; Swaab et al., 1986). This might at least partly explain the inconsistent results obtained using this therapy (see Jolles, 1986b).

There are also some general considerations that make 'neurotransmitter substitution' an enterprise with only a limited chance of success, one of them being the heterogeneous way cells of a given transmitter type change during aging and in Alzheimer's disease. As has been reported for other putative neurotransmitter systems, the vasopressin 'system' in the brain does not react as a unity: homogeneous while the SON and PVN are activated under these conditions (Fliers et al., 1985b; Hoogendijk et al., 1985), the suprachiasmatic nucleus (SCN) cells degenerate to a large extent after the age of 80 and even more strongly in Alzheimer's disease (Swaab et al., 1985). Also in 34-months-old rats, the different extrahypothalamic sites of vasopressin-fiber terminations do not show overall changes with age. Areas of termination that have the bed nucleus of the stria terminalis as a source show diminished fiber densities, while other areas remain unaltered (Fliers et al., 1985a). Such differential changes in the vasopressin innervation make it very difficult to substitute vasopressin levels in one area without interfering with normal vasopressin levels in other areas. In addition, deficits have been found in many different transmitter systems in Alzheimer's disease (see above; also Francis et al., 1985), so that normalization of all the different deficits of all the different neurotransmitters throughout the brain would not seem to be a simple task to accomplish.

Apart from the above-mentioned considerations, it is not realistic to expect that one can mimic the complex and naturally occurring spatial-temporal fluctuations of a local transmitter release by means of global administration of chemical substances. In addition, one can never replace the complete integrating function of a neuron by straightforward administration of transmitter. These are some of the considerations (e.g. Swaab et al., 1986) which call for skepticism regarding the potentialities of neurotransmitter 'replacement' therapy, whether in the case of neuropeptides or for other putative neurotransmitters.

In spite of the theoretical reservations which we have concerning neurotransmitter substitution therapies, we should realize that neuropeptides may act by different mechanisms and that some positive results have been reported in Alzheimer's disease following manipulating peptidergic systems. This holds true for trials with vasopressin or its analogs, analogs of *ACTH* (cf. Jolles, 1986b) and the opiate antagonist *naloxone*, that appears to improve cognition (Reisberg et al., 1983b). On the other hand, other peptide trials have turned out negative (cf. Jolles, 1986b) and the possibility of side-effects can not be excluded. For instance, an excited state characterized by paranoid delusions, agitation, elevated pulse rate and blood pressure was induced by *DDAVP* in a young woman with profound Alzheimer's disease (Collins et al., 1981). The interesting observation that *oxytocin* increases life-span in rats (Bodanszky and Engel, 1966) has, so far, not been followed up in the literature.

Miscellaneous therapies

Numerous investigations deal with the possible effects of vitamin preparations in the treatment of geriatric patients with mental impairment.

Nicotinic acid has not proved to be of value (Wittenborn, 1981).

On the basis of the presumptions that zinc deficiency would result in a vitamin B12 deficiency, which in turn would lead to dementia, a combined parenteral therapy of *vitamin B12* with *zinc*-DL-*aspartate* has been administered to Alzheimer patients and has been claimed to be effective in preventing senile dementia (Van Tiggelen et al., 1983). There is at present neither a theoretical framework for such a presumption nor any well-designed clinical trial giving support to this idea (Wittenborn, 1981; WHO, 1981; Ned. T. Geneesk., 1983). However, sellings of the vitamin preparations have gone up following Van Tiggelen's claim.

Vitamin E (alpha-tocopherol) would lower lipofuscin concentration in the mouse brain (Kruk and Enesco, 1981). There is, however, no indication that this compound, that also would act as

vasodilator, is effective against dementia (Ban, 1978).

Assuming a causal relationship between Pick's disease, Alzheimer's disease and a disturbance of zinc metabolism, *EDTA* has been given to demented patients (Richard et al., 1978). This therapy is also of interest since aluminum has been implicated in the etiology of Alzheimer's disease (Crapper McLachlan and Van Berkum, 1986). However, aluminum is tightly bound to DNA in neuronal nuclei, a binding that cannot readily be reversed. Anti-aluminum treatments should thus ideally be directed towards coupling aluminum before it gains access to neuronal nuclei in the first place. There are, in addition, at present no compounds that specifically bind aluminum. Although some practitioners claim beneficial effects of 'chelation' in Alzheimer patients, these have been anecdotal reports (with one exception), which may therefore be misleading (Shore and Wyatt, 1982). We thus have to wait for some methodologically sound studies.

Conclusion

In conclusion, 'therapeutic' drugs are at present more often the cause of pseudodementia than they are the cure of Alzheimer's disease. Consequently, there seems to be some truth in the cynical point of view concerning the treatment of old, mentally impaired patients in Shem's 'The House of God' (1984): "the cure is the disease" and that "to deliver *no* medical care is the most important thing you can do". If a patient develops symptoms of dementia, the doctor should first see whether he is not prescribing something that in fact may be causing it. A subsequent thorough clinical investigation should reveal possible pseudodementia (Millard, 1984; Van Crevel, 1986). It is characteristic for the current state of therapeutics that the only dementias for which clinically significant therapeutic results can be obtained, are the pseudodementias. For Alzheimer's disease the conclusion, surely, must be that although some of the available pharmacological therapies may improve the condition a little, there is no indication that any of these treatments can really stop or reverse the disease process. If (as has often been claimed, but so far never proven) a treatment were merely to slow down the progressive deterioration rather than 'cure' the patient, one might even ask if a prolonged suffering is really what we should aim for. Changes in the nutrition and social environment of the Alzheimer patient may be just as effective as pharmacological therapies (Held et al., 1984; Mirmiran et al., 1986). Indeed, beneficial effects from mere participation in the clinical trials are often found, but never emphasized in the placebo group of a clinical trial (e.g. Chierichetti et al., 1981). Pharmacological therapies may, in addition, produce considerable side-effects (cf. Millard, 1984) that have not been scrutinized so far.

A specific strategy for developing therapeutic tools in the Alzheimer's disease treatment has not been available until now. On the contrary, new insights and developments in brain research have simply been applied to Alzheimer patients. Many such therapies have statistically significant but clinically insignificant effects. One may wonder whether the small effects produced by quite different drugs and non-pharmacological therapies cannot best be explained, not so much by specific effects, but rather by a generalized stimulation of the brain. Recent observations, both from our own group and from others give support for such a possibility. The vasopressin neuron in the SON and PVN, probably osmotically stimulated secundary to a loss of binding sites in the kidney, remains capable of increased neurosecretion in senescence, both in rats and in human subjects (Fliers and Swaab, 1983; 1986). In contrast, the vasopressin cells of the SCN degenerate after the age of 80, and even more pronounced in Alzheimer's disease (Swaab et al., 1985).

The idea that symptoms of aging (and dementia) may be due to a decrease in neuronal stimulation, is not a new one. Lorand (1913) already stated: "work of any kind, even mental work alone, is means of preventing precocious senility". Dietary restriction, an effective way to increase the life-

span of rodents, might also work by stimulating the animals in a generalized way (Zoler, 1984). 'Compensatory' dendritic outgrowth is normally occurring in senescence (Buell and Coleman, 1979, 1981). A similar process is stimulated by environmental factors in the adult rat (Uylings et al., 1978; Mirmiran et al., 1986). Since no such 'compensatory' dendritic outgrowth occurs in Alzheimer's disease, a key-question for an effective prevention or therapy in Alzheimer's disease may thus be how to effectively stimulate the various neuronal systems that are most vulnerable in this condition. On the other hand, if Maurice Ravel really had Alzheimer's disease (Dalessio, 1984), then neither a productive life, nor a family stimulating him (e.g., by taking him on frequent trips) prevented or cured him from the disease. Moreover, not every change in environment needs to be beneficial for the Alzheimer patient. These patients may, in fact, be so vulnerable to changes in the environment that the effects of the preparatory workup before inclusion of such patients in a trial might already have adverse effects on them (Etienne et al., 1981).

For the time being, Millard's advice (1984) might be the best: "Until better evidence is available I think I shall tell my mother to go on doing the crossword: like other organs may not brains deteriorate with disuse?" The lack of therapeutic success clearly underlines the need for fundamental research on aging of the brain and on Alzheimer's disease. At present, this seems to be the only way ever to arrive at a rational strategy for the treatment of this condition.

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Discussion

J. M. RABEY: It still needs to be established if the dying system of the brain works by the principle of all or nothing or there is still a period when the cells are still alive but sick and therefore can be helped by manipulation at the presynaptic level. A good example is the treatment of Parkinson's disease. For years we may have succeeded in treating Parkinson patients, improving their performance until there is a complete failure of the system.

ANSWER: That is true, but I think that also these therapeutic effects in Parkinson's disease are in support of my thesis that little may be expected if transmitters are used to substitute for cell loss. Therapy in Parkinson's disease, although initially effective, becomes at a certain moment ineffective. That means that the precursor (L-dopa) cannot replace the death neurons. It can only help the surviving neurons. However, the biochemistry of the dopaminergic neuron has the advantage of being able to profit from precursor that is administered. This cannot be expected to a similar degree from other transmitter systems (e.g. peptides). Cell loss is the hallmark of dementia and it affects many different brain neurotransmitters, so that I am sceptical about the substitution potentialities.

F. BROWN: Please clarify the statement about neuroleptic therapy causing 'dementia'.

ANSWER: Quite frequently old subjects are brought into the clinics with the diagnosis 'dementia'. The condition appears, however, to be due to misuse of medicines. When the various medicines they got, often from different physicians, are stopped, they are cured within a short time. Neuroleptics are often the cause of such pseudodementias (De Beer and Simons, 1977).

D. M. GASH: In trying to interpret the significance of the loss

of small vasopressin neurons in the suprachiasmatic nucleus (SCN) in human aging as compared to the relative stability of large magnocellular supraoptic and paraventricular neurons (SON and PVN) could other principles be operating than those suggested? For example, could those neurons generated earlier in development be more stable during aging? One could also suggest other possibilities, such as the phylogenetically older neurons being the last to undergo aging. Have you evaluated these alternate explanations?

R. M. TERRY: *Comment*: Dr. Gash suggested small neurons particularly lost in normal aging neocortex. Although Brody claimed that in 1955, data from Haug's lab. and my own are quite opposite (cf. Terry, 1986). Large neurons decrease, small neurons are preserved.

ANSWER: Our own work shows also that one cannot simply predict cell death from cell size. In the human hypothalamus the SON and PVN cells are the largest and the most stable (Fliers et al., 1985; Fliers and Swaab, 1986). The SCN cells start to degenerate after the age of 80. These cells are the smallest. However, intermediate in size, the cells of the sexual dimorphic nucleus of the preoptic area (SDN) show the earliest degeneration. A cell loss was found from the age of 40 onwards in this nucleus (Swaab and Fliers, 1985). But, concerning the cause of cell death and cell stability all possibilities are still open. The only way to give a real answer to Dr. Gash's questions is to do the type of experiments we plan to do in the near future, i.e., activate or inhibit the neurons for a long time during aging and see whether we will influence in that way cell stability or degeneration.

A. GOWER: Could you comment further on the use of the 'nootropic' piracetam in Alzheimer's disease, particularly as several drug companies are busy developing similar compounds.
ANSWER: In controlled studies it was inactive in Alzheimer patients (Diesfeldt et al., 1978; Wittenborn, 1981) and it is currently marketed for use in transient ischemic attacks.

J. E. PISETSKY: Have antiviral agents been used? Has nicotinic acid not been used as vasodilator but to reduce cholesterol?

ANSWER: To my knowledge nobody has used antiviral drugs in Alzheimer patients, and there is no positive effect on dementia reported from nicotinic acid (Ban, 1978; Wittenborn, 1981).

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EXHIBIT 29

Activation of neurotransmitters in the brain: strategies in the treatment of AD/SDAT

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Brains from people with pre-mortem symptoms of Alsheimer's disease (AD/SDAT) characteristically exhibit major reductions in cortical choline acetyltransferase (CAT) activity suggesting parallel losses of the long-axon cholinergic neurons that run from the basal forebrain and septum to the cerebral and hippocampal cortices. Other neurotransmitters may also be deficient in such brains, especially from patients whose disease started at a relatively young age and was characterized by a particularly rapid course: Somatostatin levels in the cerebral cortex may be markedly reduced, as may those of norepinephrine within the *locus coeruleus* and cortical regions innervated by this nucleus. Just the same, the magnitude of the cholinergic deficit, its ubiquity in afflicted brains, and the frequency with which the cholinergic deficit is the only major neurochemical change noted, all encourage the belief that a pharmacologic manipulation that partially restores the putative deficiency in cholinergic neurotransmission might provide the basis for a useful therapeutic strategy for this tragic and otherwise ultimately-fatal disease.

Numerous investigators are attempting to explore this possibility, probably encouraged by the example of Parkinson's disease: Although that disorder is associated with deficiencies in a number of brain neurotransmitters, drugs that enhance the release or mimic the actions of just one such compound — dopamine, the one most characteristically disturbed — can be of very major benefit to Parkinsonian patients. At present, not a single drug clearly established as enhancing central cholinergic neurotransmission has been approved for use in the United States, a situation that contrasts sharply with, for example, the availability of drugs that enhance dopamine-mediated or serotonin-mediated neurotransmission. Hence, a major problem for investigators wishing to explore this treatment strategy is having something to test. As described below, this problem is being addressed in various institutions by generating new compounds; by preparing known compounds (like phosphatidylcholine [PC]) in adequately-pure and palatable forms; and by

conducting research showing that available drugs (like piracetam) not previously associated with cholinergic synapses might actually be acting by facilitating cholinergic transmission.

Some of the theoretical loci at which a drug might act to enhance cholinergic neurotransmission, and thereby possibly benefit patients with AD/SDAT, include the following:

1. As *agonists* on *post-synaptic,* presumably muscarinic brain receptors (e.g., in the hippocampal or cerebral cortices). The utility of this strategy requires that muscarinic receptors be heterogeneous, and that the particular receptors that are located post-synaptically to degenerated cholinergic terminals and that respond to the agonist be different from muscarinic receptors at other brain loci and elsewhere in the body. Otherwise, the generalized activation of muscarinic receptors would cause unacceptable side-effects. No information is available concerning the possibility that a unique family of post-synaptic receptors exists in the cortices, nor have cholinergic agonists been described which act solely in these brain regions.

2. As *antagonists* of muscarinic receptors located *pre-synaptically,* on surviving cortical or hippocampal cholinergic terminals. Activation of these receptors would be expected to diminish the amount of acetylcholine released from the terminals per firing; blockade of the receptors would, similarly, be expected to increase acetylcholine release per firing. Drugs like atropine or scopolamine do, in fact, augment the quantities of acetylcholine released when cholinergic neurons (in superfused striatal slices) are depolarized by electrical stimulation; however, they also block the post-synaptic effects of the acetylcholine, which renders them useless for enhancing cholinergic neurotransmission. As discussed above, the utility of this strategy will require that Nature have endowed the brain and body with a variety of distinct populations of muscarinic receptors, and that those existing on pre-synaptic cholinergic terminals be responsive to antagonists which have no effects on muscarinic receptors elsewhere. Some evidence is available that this may be the case, and that post-synaptic muscarinic receptors (perhaps the same as those termed "M1" receptors) differ pharmacologically from the pre-synaptic variety (termed "M2"); however, no drugs have been identified that antagonize the latter without also having a substantial effect on the former.

3. As *Acetylcholinesterase inhibitors,* which act either within the synapse — to prolong the survival of released acetylcholine — or within the pre-synaptic terminal — to increase the amounts of stored transmitter available for release per firing. Here also, the problem is one of specificity: A drug that inhibited acetylcholinesterases everywhere, as physostigmine apparently does, and thus enhanced cholinergic transmission everywhere, would have too many side-effects to be used clinically. (One strategy — discussed elsewhere in this volume — that attempts to circumvent this toxicity problem utilizes

physostigmine in combination with an agent, supplemental choline or lecithin, thought to enhance acetylcholine synthesis.) If the acetylcholinesterase enzymes are biochemically heterogeneous, such that the acetylcholinesterase present in the vicinity of cortical cholinergic synapses differs from that associated with, for example, vagus nerve synapses, then it may be possible to design drugs that affect the former but not the latter enzyme. At present, no such drugs are known.

4. Pre-synaptically, to enhance the spontaneous or depolarization-induced *release of stored acetylcholine.* Such agents might act, for example, by increasing the entrance of calcium into cholinergic terminals upon depolarization, or by slowing the uptake of this calcium into synaptic vesicles or other intracellular membranes. At present, no agents have been identified which selectively raise calcium levels within cholinergic terminals, much less the levels within just those terminals that are located within the cerebral cortex and hippocampus.

If an agent could be identified that acted, without unacceptable side-effects, to enhance the release of acetylcholine from cortical terminals (e.g., by raising calcium levels, or by any of the other mechanisms discussed in this report), it would seem prudent that its administration be coupled with a treatment capable of causing a parallel increase in acetylcholine *synthesis,* lest the transmitter rapidly become depleted within the terminals. A sustained increase in acetylcholine synthesis, would, in turn, probably require that the terminals be provided with sufficient supplemental choline to prevent depletion of this precursor or, perhaps worse, from sustaining their free choline levels by accelerating the hydrolysis of choline-containing phospholipids (like lecithin, or phosphatidylcholine [PC]) in their own membranes. Similar arguments can be adduced for the wisdom of providing a supplemental source of choline to patients receiving acetylcholinesterase inhibitors, like physostigmine (discussed above): These drugs, by slowing the hydrolysis of intra-synaptic acetylcholine, diminish the amount of free choline available for re-uptake into the terminal and for acetylation back to acetylcholine; their administration thus risks depleting the terminals of their free choline and accelerating the hydrolysis of membrane phospholipids.

5. Pre-synaptically to *enhance acetylcholine synthesis,* by providing the nerve terminal with *supplemental choline or acetyl-CoA.* There is abundant evidence that the enzyme choline acetyltransferase (CAT) is unsaturated with both of its substrates in vivo, and that the amounts of acetylcholine synthesized in, and released from, cholinergic terminals are enhanced when the brain is supplied with supplemental choline (Fig.). For human subjects, this is most readily done by administering a choline-containing compound like PC orally; this treatment sequentially elevates choline levels in the plasma and cerebrospinal fluid.

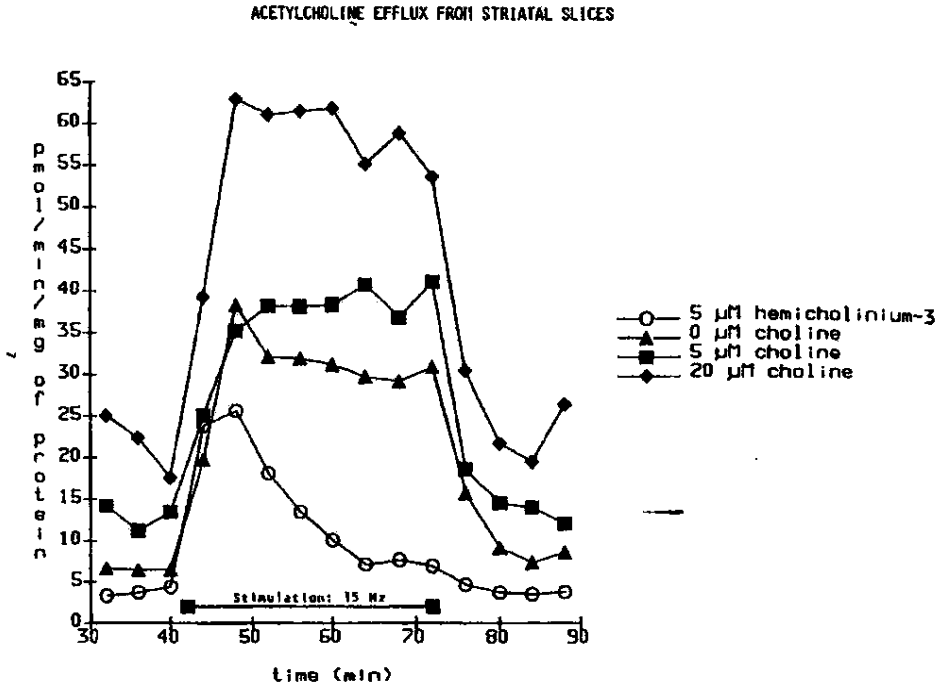

Fig. — Release of acetylcholine from rat striatal slices superfused in the presence of various concentrations of exogenous choline or hemicholinium-3 (HC-3). The bar indicates the stimulation period. Individual points are means of 3-4 separate experiments. (S.D. ranged between 5 and 15% of the values.) Choline concentrations in the physiologic range (5-20 micromolar) affected the quantities of acetylcholine released basally and with stimulation. Although the release of acetylcholine during stimulation was sustained even when free choline was lacking in the medium ("0 micromolar"), addition of HC-3 markedly reduced acetylcholine release. This suggests that the choline molecules used to sustain acetylcholine release (at 0 micromolar choline) were obtained by hydrolyzing the PC in neuronal membranes; the choline in the PC — which faces outward, into the synapses — is then taken back up into the cholinergic terminal by a high-affinity uptake mechanism which is blocked by HC-3. From: Maire, J.C., Blusztajn, J.K. and Wurtman, R.J. (1984). Effects of exogenous choline on acetylcholine and choline contents and release in striatal slices. In: *Dynamics of Cholinergic Function*, I. Hanin (ed.). Plenum Press, New York (in press).

Attempts to treat AD/SDAT with supplemental choline or PC for brief periods have not, in general, met with success. However, in the one study (R. Levy *et al.*, personal communication) that provided highly-purified (95%) PC for long periods (6 months of treatment, followed by a 6-month washout period) using a double-blind protocol, a large subgroup of patients was identified whose members exhibited significant improvement in cognitive functions and self-care indices. (This subgroup exhibited smaller increases in plasma choline levels than those occurring in the larger group of non-responders; they also tended to be somewhat older.) This finding awaits confirmation. Besides possibly increasing the synthesis and release of acetylcholine, supplemental choline (or PC) might also benefit some patients with AD/SDAT by slowing the hydrolysis of choline-containing membrane phospholipids, as discussed above.

6. By *activating excitatory receptors on the dendrites or perikarya of surviving cholinergic neurons,* and thereby accelerating the firing frequencies of the neurons. Identification of such drugs probably requires the availability of some information concerning the types of receptors present on cholinergic neurons in the septum and basal forebrain, space and, by implication, the identities of the neurotransmitters that normally impinge on these neurons. Two probable examples of such transmitters are the peptides ACTH and beta-endorphin, both of which are present in, and presumably released from, terminals of a peptidergic tract running from the hypothalamus to the septum. Installation of synthetic ACTH within the septum (or the cerebrospinal fluid) is followed by depletion of hippocampal acetylcholine, without changes in hippocampal choline levels — a pattern compatible with the hypothesis that the ACTH, an excitatory compound, acts by accelerating the firing of the septo-hippocampal cholinergic neurons. Installation of beta-endorphin, an inhibitory transmitter, has opposite effects on hippocampal acetylcholine levels (and, presumably, on acetylcholine release from the terminals). One could thus imagine enhancing the relase of acetylcholine from surviving septo-hippocampal cholinergic neurons by administering a drug that crosses the blood-brain barrier (unlike ACTH or related peptides) and acts either as an agonist at post-synaptic ACTH receptors, or — like naloxone — as an antagonist at beta-endorphin receptors.

Piracetam and other nootropic drugs may act, in part, via a similar mechanism: Administration of piracetam to rats reduces hippocampal acetylcholine levels, suggesting that it accelerates the release of the transmitter to a rate surpassing acetylcholine's synthesis. Conceivably, the drug may assume, within the brain, a conformation resembling that of ACTH or of another excitatory compound for which post-synaptic receptors exist on septo-hippocampal neurons. The administration to aged rats of a combination of piracetam and choline reportedly diminishes the tendency toward age-related memory loss. This treatment might be expected to enable the neurons

to sustain acetylcholine synthesis in the face of a continued acceleration in firing. Piracetam may also positively affect the blood flow and energy metabolism of the brain; this might be expected to facilitate cholinergic transmission in AD/SDAT by increasing the production of acetyl-CoA within nerve terminals or perhaps by restoring the uptake of "free" choline from the synaptic cleft into the pre-synaptic terminal. It would be interesting to determine whether piracetam affects the spontaneous or stimulus-induced firing of cholinergic neurons in the septo-hippocampal tract *in vitro*, or whether nootropic agents interact with receptors for any of the compounds that may act as transmitters on cholinergic neurons in the septum or basal forebrain.

7. by influencing the *interactions of adenosine with its pre- or post-synaptic receptors.* Adenosine may be released from brain neurons as a *bona fide* neurotransmitter, as a co-transmitter (e.g., liberated from the ATP stored along with catecholamines), or simply as a by-product of intermediary metabolism, especially when oxygenation has been less than adequate. The purine is pharmacologically-active at very many synapses, both pre-synaptically and post-synaptically; it can inhibit the release of, and receptor responses to, a variety of neurotransmitters. It can also produce excitatory effects, for example, markedly potentiating the increase in blood pressure caused by giving nicotine. The ubiquity of adenosine's actions, and the likelihood that its release would be enhanced by processes known to accompany AD/SDAT (like diminished blood flow and decreased glucose utilization) would seem to mark it as an especially good candidate locus for designing new drugs to modulate neurotransmission in this disease group.

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WURTMAN, R.J. (1985). Alzheimer's disease. *Scientific American* 252: 62-74.

NORMAL AGING, ALZHEIMER'S DISEASE AND SENILE DEMENTIA

ASPECTS ON ETIOLOGY, PATHOGENESIS, DIAGNOSIS AND TREATMENT

Chief Editor: C.G. Gottfries

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